UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE CIÊNCIAS BIOLÓGICAS

DOUTORADO EM BIOQUÍMICA E FISIOLOGIA

Investigação de Distúrbios Metabólicos Associados à Hiperuricemia; Atividades Biológicas de *Myrciaria cauliflora, Crataeva tapia e Indigofera suffruticosa*

TIAGO FERREIRA DA SILVA ARAÚJO

Orientadora: Profa. Dra. Vera Lúcia de Menezes Lima

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TIAGO FERREIRA DA SILVA ARAÚJO

Investigação de Distúrbios Metabólicos Associados à Hiperuricemia; Atividades Biológicas de *Myrciaria cauliflora, Crataeva tapia e Indigofera suffruticosa*

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"The greatest results in life are usually attained by simple means and the exercise of ordinary qualities. These may for the most part be summed in these two: common-sense and perseverance".

Owen Feltham

Aos meus pais, Antônio Araújo e Terezinha Ferreira,

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RESUMO

O presente estudo teve como objetivo investigar a relação entre hiperuricemia, distúrbios metabólicos e as atividades biológicas de *Myrciaria cauliflora*, *Crataeva tapia* e *Indigofera suffruticosa*. Para tanto, foi realizado inicialmente um estudo populacional, com 3620 voluntários, adultos, homens, não diabéticos, do Nordeste brasileiro. Obesidade abdominal e hipertrigliceridemia foram avaliadas para a identificação do fenótipo denominado Cintura Hipertrigliceridêmica (CHTG) e para a avaliação da influência desses distúrbios metabólicos sobre a hiperuricemia. Posteriormente, estudos com modelo animal (*Mus musculus*) foram conduzidos. Assim, extratos orgânicos (etéreo, acetônico e metanólico) foram preparados a partir do epicarpo de frutos maduros de *M. cauliflora*, denominados, sequencialmente, de MCEE, MCAE e MCME, nas concentrações de 200mg/Kg e de 400mg/Kg. Análise fitoquímica e estudo da toxicidade oral desses extratos foram realizadas. MCAE foi administrado durante 14 dias em camundongos com diabetes induzida por aloxana; e avaliação do perfil glicídico, lipídico, de função renal e hepática e análise histológica do pâncreas foram realizadas. Atividade anti-hiperuricêmica de MCAE, em modelo de oxonato de potássio, também foi investigada. MCEE, MCAE e MCME foram usados para a avaliação de: atividade anti-inflamatória, usando os modelos de edema de pata e de peritonite; atividade antinociceptiva, nos modelos de dor induzida por ácido acético e de placa quente; atividade antioxidante, por ensaio com 2,2-difenil-β-picrilhidrazil; e atividade antitumoral, contra tumor sólido de carcinoma de Ehrlich. Lectina de *C. tapia* foi purificada e testada para avaliação de atividade hipoglicêmica. Extratos etéreo, clorofórmico e acetônico de folhas de *I. suffruticosa* foram preparados, analisados fitoquimicamente e testados contra cepas de *S. aureus*. Como principais resultados, este estudo demonstrou que: obesidade abdominal e hipertrigliceridemia, isoladas, mostraram significativas razões de chance (RC) sobre a presença de hiperuricemia, porem o fenótipo CHTG demonstrou o maior efeito (RC = 4,3), especialmente após o uso dos pontos de corte obtidos especificamente para a população do estudo; hiperuricemia apresentou uma forte associação com alto risco de morte por evento cardiovascular em dez anos (RC = 3,5); 200mg/Kg/dia e 400mg/Kg/dia de MCAE causou uma redução significativa da glicose plasmática e redução nos níveis séricos de triglicerídios, uréia, creatinina e transaminases, aumento de HDL-colesterol, melhora do aspecto morfológico das ilhotas pancreáticas e diminuição de cerca de 50% nos níveis de ácido úrico; MCEE, MCAE e MCME apresentaram relevante atividade antioxidante e produziram reduções significativas da resposta inflamatória, de nocicepção e da massa tumoral; lectina de *C. tapia* provocou redução significativa nos níveis de glicose, melhora das funções e dos aspectos morfológicos dos rins, pâncreas e fígado de camundongos diabéticos; o extrato acetônico de folhas de *I. suffruticosa* foi um potente inibidor de *S. aureus*, seguido pelo extrato clorofórmico, melhorando também sinergisticamente o efeito da eritromicina. Portanto, hiperuricemia está bastante relacionada com o fenótipo CHTG em homens do Nordeste do Brasil, podendo elevar em muito o risco cardiovascular desses indivíduos. *M. cauliflora* demonstrou um grande potencial terapêutico para hiperuricemia e as condições metabólicas associadas e, assim como lectina de *C. tapia*, demonstrou ser um agente promissor para o tratamento da diabetes; enquanto que *I. suffruticosa* mostrou-se ser bastante promissora contra *S. aureus*.

Palavras-chaves: Hiperuricemia, Distúrbios Metabólicos, Cintura Hipertrigliceridêmica, Risco Cardiovascular, Atividades Biológicas de Plantas.

ABSTRACT

This study aimed to investigate the relationship among hyperuricemia, metabolic disorders and the biological activities of *Myrciaria cauliflora*, *Crataeva tapia* e *Indigofera suffruticosa*. Therefore, it was initially performed a population study, with 3620 volunteers, adults, men, non-diabetic, from northeastern Brazil. Abdominal obesity and hypertriglyceridemia were evaluated for the identification of phenotype referred as Hypertriglyceridemic Waist (HTGW) and to assess the influence of these metabolic disorders hyperuricemia. Later, animal model studies (*Mus musculus*) were conducted. Thus, organic extracts (ether, acetone, and methanol) were prepared from ripe fruit epicarp of *M. cauliflora* referred to, sequentially, MCEE, MCAE, and MCME, at concentrations of 200mg/Kg and 400mg/Kg. Phytochemical analysis and study of oral toxicity of these extracts were made. MCAE was administered for 14 days in mice with alloxan-induced diabetes; and evaluation of glucose profile, lipid, renal and hepatic function and histological analysis of the pancreas were performed. Anti-hyperuricemic activity of MCAE, in potassium oxonate model, was investigated. MCEE, MCAE, and MCME were used for evaluation of: anti-inflammatory activity, using the rat paw edema model and peritonitis; antinociceptive activity, in models of pain induced by acetic acid and hot plate; antioxidant activity, by 2,2-diphenyl-β-picrylhydrazyl assay; and antitumor activity against solid tumor of Ehrlich carcinoma. Lectin of *C. tapia* was purified and tested for evaluation of hypoglycemic activity. Ether, chloroform and acetone extracts of leaves of *I. suffruticosa* were prepared, phytochemically analyzed and tested against strains of *S. aureus*. As main results, this study demonstrated that: abdominal obesity and hypertriglyceridemia, isolated, showed significant odds ratios (OR) for the presence of hyperuricemia, but HTGW phenotype demonstrated the most effect (OR = 4.3), especially after use the cutoffs obtained specifically for the study population; hyperuricemia showed a strong association with high risk of cardiovascular events death in ten years (OR = 3.5); 200mg/Kg/dia and 400mg/Kg/dia of MCAE caused a significant reduction in plasma glucose and reduction in serum levels of triglycerides, urea, creatinine and transaminases, increase in HDL-cholesterol, improvement in the morphological appearance of the pancreatic islets and decrease about 50% in the levels of uric acid; MCEE, MCAE, and MCME presented significant antioxidant activity and produced significant reductions in the inflammatory response, nociception and of the tumor mass; *C. tapia* lectin caused a significant reduction in glucose levels, improved the function and morphology of the kidneys, pancreas and liver of diabetic mice; and the acetone extract from the leaves of *I. suffruticosa* was a potent inhibitor of S. aureus followed by chloroform extract, also synergistically improving the effect of erythromycin. Therefore, hyperuricemia is closely related to the HTGW phenotype in men in Northeast Brazil, and can significantly increase the cardiovascular risk of these individuals. *M. cauliflora* demonstrated a great therapeutic potential for hyperuricemia and associated metabolic conditions, as well as *C. tapia* lectin proved to be a promising agent for the treatment of diabetes; while *I. suffruticosa* shown to be quite promising against *S. aureus*.

Keywords: Hyperuricemia, Metabolic Disorders, Hypertriglyceridemic Waist, Cardiovascular Risk, Plant Biological Activities.

I. INTRODUÇÃO

O equilíbrio físico-químico de um organismo vivo é denominado homeostase e sua manutenção **se** dá através de diversas reações químicas que envolvem tanto vias anabólicas quanto catabólicas. Nos animais, a manutenção da homeostase orgânica é dependente da excreção de compostos tóxicos. Estas substâncias tóxicas são denominadas produtos de excreta e podem ser eliminadas do organismo através dos fluidos biológicos corporais, principalmente através da urina. Os compostos nitrogenados fazem parte destas substâncias que necessitam ser excretadas em diferentes animais, sendo eles: a amônia, a uréia e o ácido úrico (OLIVEIRA et al., 2001).

O ácido úrico (AU) é um ácido orgânico sintetizado pelos mamíferos como produto do metabolismo das bases púricas nitrogenadas adenina e guanina. Em animais mamíferos, exceto seres humanos e alguns primatas, o ácido úrico necessita ainda ser degradado em alantoína pela ação catalítica da enzima hepática uricase. Contudo, o gene que codifica a uricase sofreu silenciamento mutacional durante a evolução humana e como consequência desta inativação, os níveis sanguíneos de ácido úrico são muito maiores em seres humanos que em outros mamíferos. Desta forma, em seres humanos o ácido úrico é considerado o produto final da degradação destas bases nitrogenadas e encontra-se em pH fisiológico na forma de urato monossódico, apresentando um *pKa* de 5,75 (SO e THORENS, 2010; ODA et al., 2002).

O excesso de ácido úrico caracteriza o distúrbio metabólico hiperuricemia (HU). O desenvolvimento de HU pode acontecer através de duas vias bioquímicas: o aumento da biossíntese hepática e/ou a diminuição da depuração renal. Desta forma, quando os níveis de AU excedem 6,0 e 7,0 mg/dL em mulheres e em homens, respectivamente, tem-se a caracterização laboratorial e confirmação do diagnóstico da HU. Estas concentrações correspondem ao limite da solubilidade dos uratos monossódicos e, por isso, durante a hiperuricemia há a supersaturação de uratos e consequente propensão à sua cristalização e deposição nos tecidos (RICHETTE e BARDIN, 2010; EKPENYONH et al., 2014).

Durante muito tempo as principais consequências patológicas associadas à hiperuricemia relacionavam-se à deposição dos cristais de ácido úrico nas articulações, promovendo a gota, ou no tecido renal, promovendo a urolitíase e a nefropatia por uratos.

No entanto, a elevação dos níveis de ácido úrico tem sido reportada como um dos mais novos e importantes fatores associados ao desenvolvimento de alterações metabólicas que estariam relacionadas ao aumento do risco cardiovascular. Não obstante, estudos recentes têm sugerido um papel direto do ácido úrico na fisiopatologia da doença cardiovascular (DCV), sobretudo, na deposição e calcificação da placa aterosclerótica de gorduras e agravamento da doença arterial coronariana (DAC) (MAYER et al., 2014; GOLÇALVES et al., 2015; PEREZ-RUIZ e LIOTÉ, 2007).

No caso direto às DCVs, consideradas como a maior *causa mortis* da sociedade atual, mais prevalente e mais dispendiosa, as informações científicas ainda são díspares na correlação entre HU e o risco cardiovascular. Outros distúrbios reconhecidos como fatores de risco cardiovascular, tais como a obesidade abdominal e a hipertrigliceridemia, isolados, ou em associação constituindo o fenótipo cintura hipertrigliceridêmica também podem contribuir para o desenvolvimento da hiperuricemia, o que também mantem dúvidas sobre a existência de uma verdadeira relação causal entre cada uma destas patologias (HSU et al., 2013; JURASCHEK et al., 2014).

O tratamento para hiperuricemia envolve medicamentos que estejam relacionados com a diminuição da síntese deste ácido ou com o aumento da sua excreção renal. Ainda, as plantas têm sido utilizadas no tratamento da hiperuricemia e de seus distúrbios correlacionados, como estresse oxidativo, dor, inflamação, câncer, diabetes e dislipidemias ou ainda no tratamento de doenças infecciosas. Neste contexto, plantas ricas em compostos fenólicos tem representado uma excelente fonte para a fitoterapia (NEWMAN et al., 2003).

A jaboticaba é o fruto da *Myrciaria cauliflora* (Mart.) O. Berg. (Myrtaceae), uma planta nativa do Brasil. O fruto apresenta um sabor doce com um pouco de acidez e eles são consumidos in natura. A casca da jaboticaba apresenta coloração escura, variando de roxo escuro a preto, e representa uma rica fonte de compostos químicos como os compostos fenólicos. Desta forma, produtos extraídos da casca do fruto de *M. cauliflora* podem auxiliar no tratamento da hiperuricemia e de seus distúrbios metabólicos relacionados (IDEMIR et al., 2005; LEITE-LEGATTI et al., 2012; WU et al., 2012; WU, LONG & KENNELLY, 2013; BORGES, CONCEIÇÃO & SILVEIRA, 2014; MERCALI et al., 2015.

Outras espécies que têm sido bastante utilizadas na medicina popular são a *Crataeva tapia* e a *Indigofera suffruticosa*. Na medicina popular a casca do tronco de *C. tapia* é usada como tônico, estomáquico, antidisentérico, febrífugo e no tratamento do

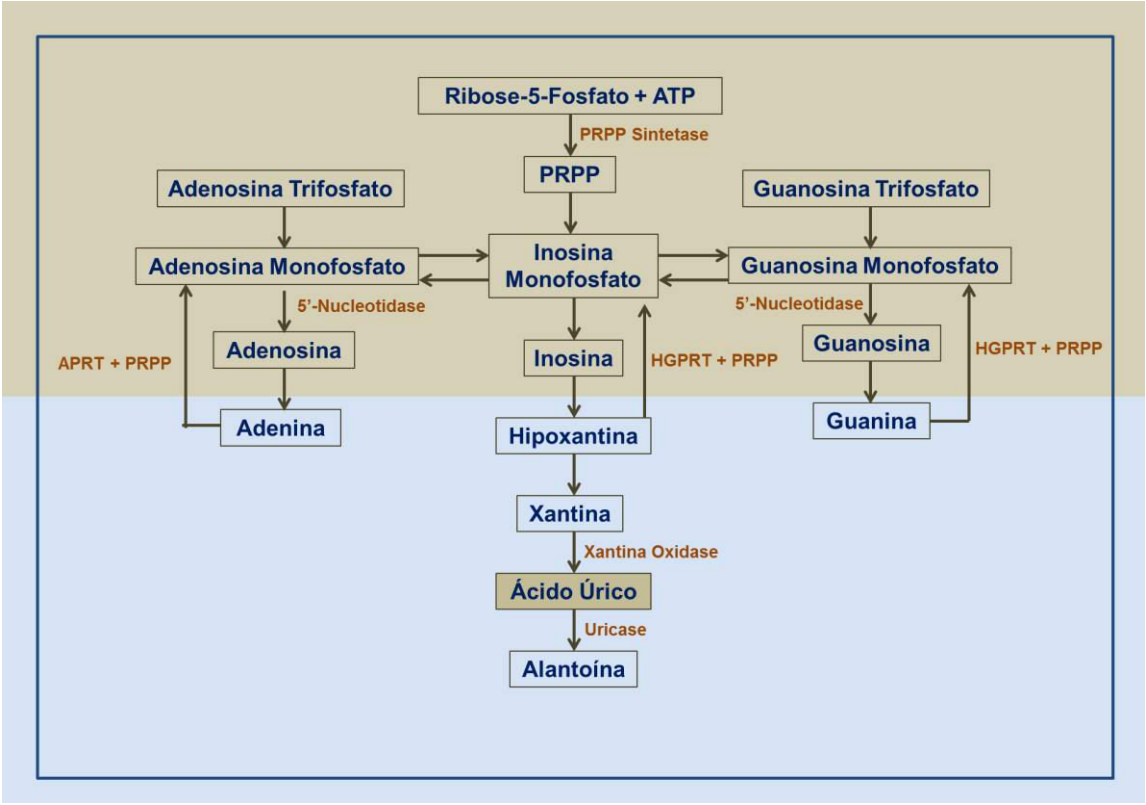
diabetes. Enquanto a *I. suffruticosa* apresenta na população tais propriedades fitoterápicas: antiespasmódicas, sedativas, diuréticas, purgativas, odontálgicas. Deste modo, com base em estudos científicos que relatam os potenciais farmacológicos de determinados compostos extraídos de plantas que são utilizadas na medicina popular para o tratamento de doenças justifica-se a necessidade de extrair e avaliar tais compostos na casca do fruto maduro de *M. cauliflora,* na folha da *I. suffrutica* e na entrecasca de *Crataeva tapia* que possam contribuir com o arsenal terapêutico.

II. FUNDAMENTAÇÃO TEÓRICA

2.1 Ácido Úrico e Desenvolvimento da Hiperuricemia

Ácido úrico é um ácido orgânico com um pKa de 5,8, que é primariamente encontrado e distribuído no fluido extracelular como urato monossódico, é o produto final da degradação das purinas em humanos (**Figura 1**) (DESIDERI et al., 2014).

Figura 1. Metabolismo do ácido úrico.



Fonte: ARAÚJO, Tiago Ferreira da Silva (2015). ATP – adenosina trifosfato; PRPP – fosforibosil pirofosfato; APRT – adenina fosforibosiltransferase; HGPRT – hipoantina guanina fosforibosiltransferase

Quando o pH é menor que 5.75, como pode ocorrer na urina, a forma predominante do ácido úrico é a não ionizada enquanto que a um pH de 7,4, circula a forma ionizada (EKPENYONH et al., 2014). Devido à elevada concentração de sódio no compartimento extracelular, ácido úrico está largamente presente como urato monossódico, com um baixo

limite de solubilidade de cerca de 6 mg / dL (360μmol / L) (RICHETTE e BARDIN, 2010). Os níveis séricos de ácido úrico são bioquímicamente equilibrados em humanos pela ausência da enzima uricase (RUGGIERO et al., 2006). Em mamíferos não-humanos, o ácido úrico continua a ser degradada em alantoína pela ação catalítica da enzima uricase fígado (SO et al., 2010). No entanto, o gene que codifica a uricase sofreu silenciamento mutacional na evolução humana e como um resultado desta inativação, níveis de ácido úrico no sangue são muito mais elevados em humanos que em outros mamíferos (ODA et al., 2002).

A hiperuricemia é geralmente definida quando os níveis de ácido úrico encontram-se maiores que 7,0 mg / dL (416μmol / L) em homens e maiores que 6,0 mg / dL (360 pmol / L) em mulheres (FENECH et al., 2014). A hiperuricemia é uma das alterações bioquímicas mais comumente encontradas na prática clínica e estudos têm relatado que ela afeta cerca de 10% dos adultos globalmente (MURUGAIYAH et al., 2009). Quando os níveis sanguíneos de ácido úrico excedem o limite de solubilidade o risco de cristalização e precipitação aumenta consideravelmente (DESIDERI et al., 2014). Neste contexto, a deposição de cristais de ácido úrico em tecidos articulares e periarticulares promove a gota. As consequências clínicas para esta doença incluem episódios de dor aguda e inflamação, devido a formação de tofos subcutâneos constituídos de ácido úrico, que podem promover uma destruição osteoarticular (PEREZ-RUIZ e LIOTÉ, 2007).

A hiperuricemia esteve, por durante muito tempo, associada principalmente ao desenvolvimento da gota (DESIDERI et al., 2014). No entanto, evidências científicas recentes demonstraram que o ácido úrico exerce uma ação pró-oxidante perigosa, exercendo papel eficaz no desenvolvimento de outras doenças (ACHARYA et al., 2015). Atualmente, é bem postulado o papel do ácido úrico na gênese da doença renal crônica (CHOU et al., 2014), da resistência à insulina (CHOI et al., 2014) e diabetes (JURASCHEK et al., 2014), desordens bipolares (ALBERT et al., 2015), na disfunção eréctil (SOLAK et al., 2014), dislipidemias (PENG et al., 2015), hipertensão arterial (BJORNSTAD et al., 2014), síndrome metabólica (SUN et al., 2014), e na calcificação da artéria coronária (GROSSMAN et al., 2014), doença aterosclerótica carotídea (MAYER et al., 2014) e outras doenças cardiovasculares e cerebrovasculares (GOLÇALVES et al., 2015)..

As elevações nos níveis séricos de ácido úrico ocorrem principalmente pela diminuição da excreção pelos rins, como ocorre quando a função renal ou fluxo sanguíneo

renal são reduzidos. Ácido úrico pode também estar aumentado por superprodução e tal evento ocorre com o aumento da ingestão de alimentos ricos em purinas, abuso de álcool ou o consumo excessivo de frutose, ou por condições associadas com elevado *turnover* de purinas (KESEBIR et al., 2014). Na **Tabela 1** estão descritas algumas das principais causas relacionadas ao desenvolvimento de hiperuricemia.

Tabela 1. Causas de Hiperuricemia

HIPERURICEMIA
Aumento da Síntese
Deficiência da hipoxantina-guanina fosforibosil transferase (parcialmente, na Síndrome de Seegmiller e completamente, na Síndrome de Lesch-Nyhan
Aumento da atividade da fosforibosil pirofosfato sintase
Consumo excessivo de alimentos ricos em bases púricas
Dieta cetogênica
Doenças de degradação de purinas (elevado *turnover* de nucleotídeos)
Doenças de rápida proliferação cellular e morte (crise blástica da leucemia, psoríases, doenças linfoproliferativas e terapia citotóxica ou quimioterapia)
Diminuição da Excreção Renal
Insuficiência renal com diminuição da taxa filtração glomerular
Diminuição da secreção tubular (cetoacidose diabética)
Aumento da reabsorção tubular (terapia diurética)
Hipertensão arterial sistêmica
Hipoperfusão tecidual
Etilismo
Consumo de dieta rica em frutose
Infecções
Obesidade

Fonte: Kesebir et al., 2014

2.2 Distúrbios Metabólicos Associados à Hiperuricemia

A hiperuricemia correlaciona-se com o aumento da produção de muitos mediadores inflamatórios. Em estudos experimentais, hiperuricemia está associada com a ativação de fosfolipase A2 (LYTVYN et al., 2015), aumento do níveis de Fator de Necrose Tumoral-alfa (TNF-alfa), interleucina-1β (IL-1β), interleucina-6 (IL-6), proteína quimioatrativa de monócitos-1 (MCP-1) (RUGGIERO et al., 2006). Estes mecanismos estão associados com exacerbação de uma resposta inflamatória nos estados hiperuricêmicos. Vários estudos baseados em populações tem sugerido o papel pro-inflamatório do ácido úrico em homens e mulheres, inclusive o ácido úrico no soro tem sido associado com o aumento da proteína C-reativa, uma proteína inflamatória de fase aguda (RUGGIERO et al., 2006). Por conseguinte, existe uma forte evidência para sugerir que hiperuricemia desencadeia vias pró-inflamatórias que contribuem para o desenvolvimento de doença aterosclerótica e cardiovasculares.

A associação entre hiperuricemia e doenças cardiovasculares e ateroscleróticas foi amplamente ignorada até os meados da década de 1950 e início da década de 1960 (FEIG et al., 2008). Desde então, estudos epidemiológicos têm demonstrado a relação entre os níveis séricos de ácido úrico e patologia cardiovascular, incluindo doença aterosclerótica carotídea (MAYER et al., 2015), doença arterial coronariana (SOLAK et al ., 2014) e infarto agudo do miocárdio (LEVANTESI et al., 2013).

A hiperuricemia é capaz de promover efeitos desfavoráveis sobre o endotélio vascular através da via de sinalização do fator nuclear kappa B (NF-kB) por interações das células endoteliais e os monócitos com aumento de expressão de molécula de adesão celular vascular-1 (VCAM-1) e molécula de adesão intracelular 1 (ICAM-1). Neste contexto, altas concentrações de ácido úrico estimula produção de quimiocinas e moléculas de adesão envolvidos na migração e adesão de leucócitos a células endoteliais. Este mecanismo pode explicar os papéis-chave na iniciação e desenvolvimento da aterosclerose (LIANG et al, 2014).

Trabalho realizado por Peng et al., (2015) sugerem que os níveis séricos de ácido úrico aumentam de forma associada com um aumento nos níveis de LDL-colesterol, triglicéridios, colesterol total e nas concentrações de apolipoproteína B. Hiperuricemia

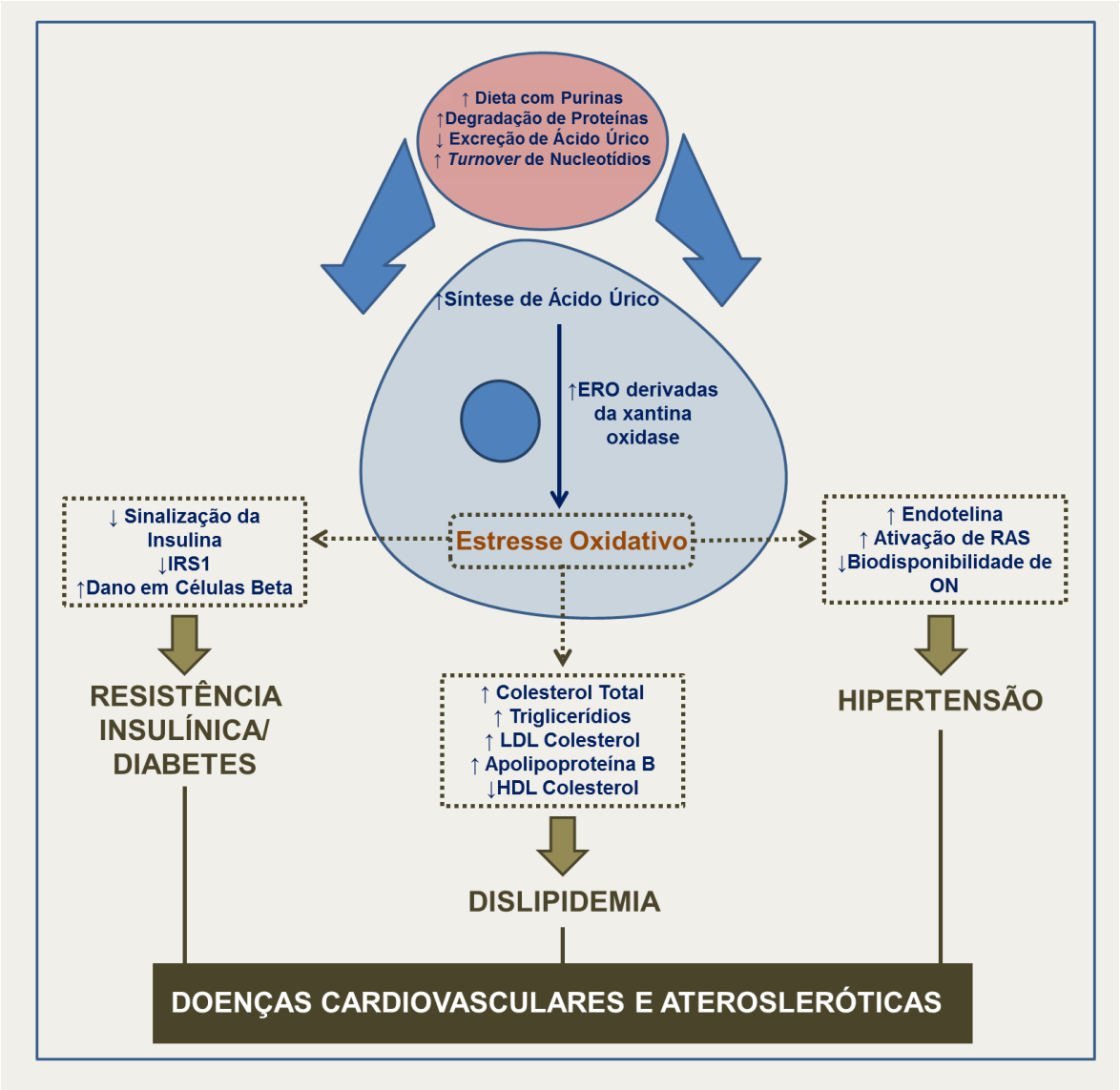
apresenta uma forte relação inversa com os níveis de HDL-colesterol. Estas dislipidemias tem uma relação estreita com a doença aterosclerótica e cardiovascular (PENG et al., 2015). Assim, a hiperuricemia pode contribuir para a etiologia da aterosclerose, estimulando-a através de duas importantes maneiras: inflamação e dislipidemia (LIPPI et al, 2011).

Além disso, a biossíntese de ácido úrico exerce efeitos deletérios em seres humanos através do aumento da produção de espécies reativas de oxigênio (ROS). A atividade da xantina oxidase é uma fonte importante da produção de ROS resultante da reação de oxidação da hipoxantina em xantina e da xantina em ácido úrico (LYVTYN et al., 2015). O estresse oxidativo têm um papel central na patogênese do diabetes. Neste contexto, o ácido úrico está emergindo como um potencial marcador de risco de diabetes (JURASCHEK et al., 2014).

Em modelos experimentais de hiperuricemia aguda (criado por tratamento com oxonato potássio) foram observadas evidências de que hiperuricemia pode induzir diretamente a resistência à insulina, um importante preditor de diabetes (ZHU et al., 2014). A insulina é uma hormônio que age sobre os receptores celulares específicos para regular a captação de glucose. A maioria dos sinais dos receptores de insulina é transmitida por mecanismos complexos que envolvem o receptor de substrato da insulina (IRS) -1/2. A presença de IRS1 humano fosforilado é um marcador molecular representante da resistência à insulina. Aumento do nível de ácido úrico pode inibir a atividade do IRS1 e induzir a resistência à insulina (ZHU et al., 2014). Estes mecanismos podem explicar a relação causal entre a hiperuricemia e o desenvolvimento de diabetes. Neste âmbito, vários estudos epidemiológicos têm relatado a possibilidade de ácido úrico promover diabetes (BHOLE et al, 2010;. BANDARU e SHANKAR, 2011; WANG et al, 2011; KATSIKI et al, 2013;. JIA et al, 2013) .

O estresse oxidativo causado por um aumento nos níveis de ácido úrico pode estar relacionada com o desenvolvimento de hipertensão. A pressão sanguínea é determinada pelo débito cardíaco, resistência periférica total, rigidez arterial e arteriolares (HSU et al., 2013). Na **Figura 2** estão demonstradas algumas vias mecanísticas relacionadas ao desenvolvimento de distúrbios metabólicos e ao aumento do risco cardiovascular.

Figura 2. Hiperuricemia e o Desenvolvimento de Distúrbios Metabólicos.



Fonte: ARAÚJO, Tiago Ferreira da Silva (2015). ERO – espécies reativas do oxigênio; IRS 1 – substrato do receptor de insulina 1; ON – óxido nítrico.

2.3 Relação Bioquímica entre Obesidade Abdominal, Hipertrigliceridemia e Desenvolvimento de Hiperuricemia

A obesidade abdominal, caracterizada pelo excesso de massa adiposa na região abdominal tem sido associada principalmente a distúrbios do metabolismo lipídico. Ácidos graxos livres encontram-se em excesso em indivíduos com obesidade abdominal, o que aumenta o aporte hepático e muscular desses lipídios, ressíntese de triglicerídios,

armazenamento ectópico dos mesmos e elevação de sua concentração sanguínea (AHMADIAN; DUNCAN; SUL, 2009).

A elevação dos níveis sanguíneos de triglicerídios é denominada de hipertrigliceridemia. Este tipo de dislipidemia pode levar a outras alterações lipídicas, pois, quando os triglicerídios se encontram em excesso, estes passam a integrar as lipoproteínas de muito baixa densidade (VLDL, do inglês *Very Low Density Lipoprotein*) em uma concentração superior à de sua constituição em um metabolismo lipídico considerado normal. Dessa forma, as VLDL tornam-se ricas em triglicerídios e, mesmo após serem catabolizadas, continuam enriquecidas neste tipo de lipídio, e originando lipoproteínas de densidade intermediária (IDL, do inglês *Intermediary Density Lipoprotein*) também ricas em triglicerídios. Estas, sequencialmente, dão origem a lipoproteínas de baixa densidade (LDL, do inglês *Low Density Lipoprotein*) com maior conteúdo de triglicerídios (CHAPMAN e SPOSITO, 2008).

À medida que o catabolismo dessas lipoproteínas modificadas ocorre, as partículas de VLDL, de IDL e de LDL ricas em triglicerídios cedem, sob a ação da Proteína Transferidora de Colesterol Éster (CETP, do inglês *Cholesterol Ester Transfer Protein*), seu alto conteúdo triglicerídico às lipoproteínas de alta densidade (HDL, do inglês *High Density Lipoprotein*), em troca de colesterol éster. Então, hipertrigliceridemia induz indiretamente alterações na estrutura e composição química das partículas de HDL e LDL (LAMARCHE et al., 1999; CHAPMAN e SPOSITO, 2008).

O complexo lipoprotéico da HDL, por não apresentar sua composição lipídica normal, passa a ser considerado, pelas células fagocíticas do sistema imune, um verdadeiro corpo estranho, o que aumenta a sua remoção da corrente sanguínea via fagocitose não mediada por receptor, promovendo a diminuição destas lipoproteínas no sangue. As partículas de HDL enriquecidas com triglicerídios tornam-se instáveis e perdem seu conteúdo protéico e passam a ser degradadas em maior quantidade nos rins, o que contribui também para a diminuição de sua concentração sanguínea. Isto é um fato que contribui em muito para o estabelecimento das placas ateroscleróticas, haja vista menores quantidades de HDL estarem exercendo o seu papel fisiológico no processo do transporte reverso do colesterol, o que aumenta o conteúdo de colesterol nos tecidos extra-hepáticos, incluindo, o tecido endotelial dos vasos sanguíneos, propiciando a formação do ateroma (LIMA et al., 2004; REAVEN, 2005; GRUNDY, 2006; KONTUSH e CHAPMAN, 2006).

Quanto às partículas de LDL ricas em triglicerídios, resultantes da ação catalítica da CETP, essas ficam menores e passam a apresentar uma densidade maior em comparação às LDL normais. Estas lipoproteínas passam a ser denominadas de LDL pequenas e densas e se tem direcionado, a esta classe de lipoproteínas, um risco de cerca de três vezes de ocorrência de infarto do miocárdio (MARUYAMA; IMAMURA; TERAMOTO, 2003). LDL pequenas e densas possuem menor afinidade pelos receptores hepáticos de LDL e, com isso, há uma diminuição de sua remoção da circulação, aumentando a probabilidade de ficarem retidas nas paredes arteriais. Estas lipoproteínas modificadas também são mais susceptíveis ao processo de oxidação, o que as fazem ser consideradas corpos estranhos, pelos macrófagos, assim como ocorre com a HDL rica em triglicerídios (GALEANO et al., 1998; KONDO et al., 2001).

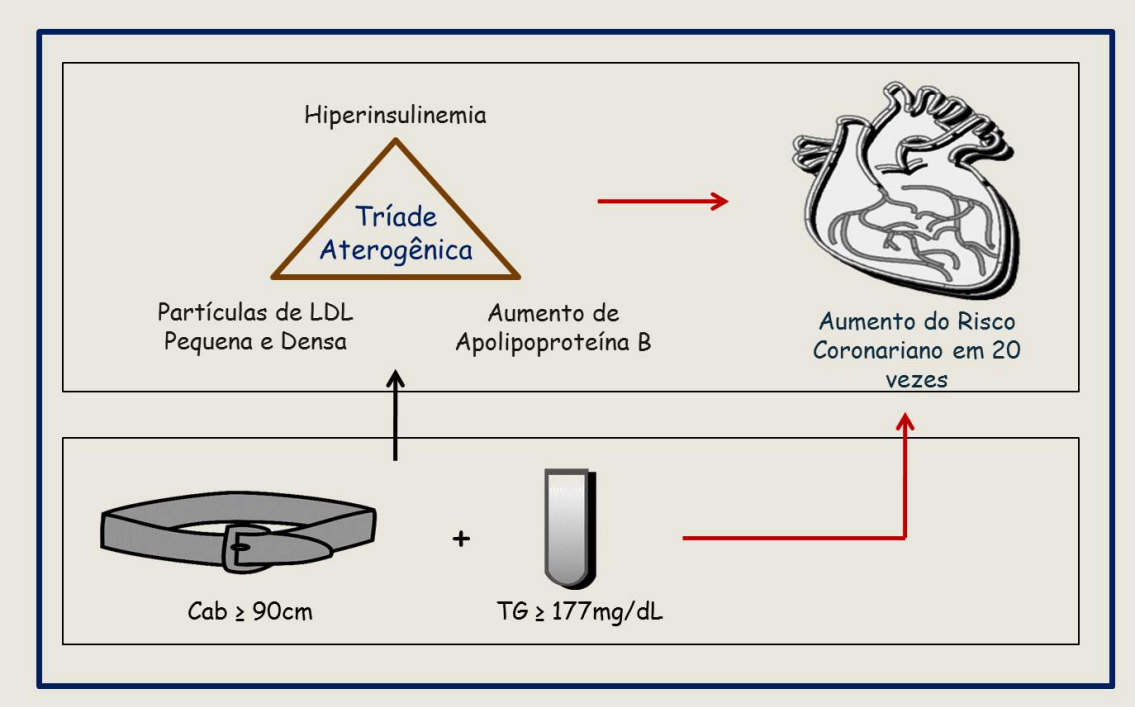
Estudos têm sugerido que altas concentrações de triglicerídios geralmente significam maiores concentrações de HDL e predominância de LDL pequenas e densas e que estas alterações lipídicas caracterizam a chamada dislipidemia aterogênica (KONDO et al., 2001; REAVEN, 2005; GRUNDY, 2006; CHAPMAN e SPOSITO, 2008). Inclusive, um parâmetro que combina triglicerídios e HDL, a razão triglicerídios/HDL-colesterol (TG/HDL-c), tem sido utilizado para identificar de forma mais simples, mas com boa acurácia, a presença de LDL pequenas e densas. Estima-se que indivíduos com uma razão TG/HDL-c maior que 0,9, adotando mmol/L como unidade de medida das concentrações destas lipoproteínas, ou maior que 2,0, quando utilizado mg/dL, apresentam partículas de LDL predominantemente maiores que 25,5 nm de diâmetro (MARUYAMA; IMAMURA; TERAMOTO, 2003).

Entretanto, mesmo mediante esta via fisiopatológica dos distúrbios lipídicos decorrentes da hipertrigliceridemia, bem como da correlação positiva sugerida entre hipertrigliceridemia e doenças cardiovasculares e ateroscleróticas, estudos têm reportado não terem encontrado esta associação e há até mesmo outros estudos que encontraram uma associação negativa. Isto é, o papel da hipertrigliceridemia com ou sem associação à obesidade abdominal, mesmo mediante um raciocínio lógico de mecanismos fisiopatológicos, pode variar de acordo com a população em estudo (AUSTIN; HANSON; EDWARDS, 1998; COUGHLAN e SORRENTINO, 2000; SUMNER et al., 2005; CRIQUI, 2007; OKOSUM e BOLTRI, 2008).

Outro ponto questionável é se a obesidade abdominal, sem a elevação dos níveis plasmáticos de triglicerídios, apresenta igual prejuízo ao metabolismo bioquímico e à integridade homeostática do indivíduo (OKOSUM e BOLTRI, 2008). Recentes estudos têm sugerido que tanto hipertrigliceridemia quanto obesidade abdominal são fatores independentes de risco cardiovascular (HAFFNER et al., 1992; DESPRÉS, 1993; BERMUDEZ e TUCKER, 2001; OKOSUN et al., 2001; BANSAL et al., 2007; NORDESTGAARD et al., 2007). Contudo, investigadores têm enfatizado que a presença associada destes distúrbios seja mesmo muito mais nociva à saúde e que, inclusive, o risco que se atribui à hiperglicemia pode ser explicado pela coexistência de hipertrigliceridemia e obesidade abdominal (DESPRÉS, 1993; OKOSUM e BOLTRI, 2008).

Em 2000, Isabelle Lemieux e colaboradores criaram a denominação fenótipo "Cintura Hipertrigliceridêmica" (HTGW, do inglês *Hypertriglyceridemic Waist*) para a presença conjunta de níveis plasmáticos de triglicerídios iguais ou superiores a 177 mg/dL e valores de circunferência abdominal maiores ou iguais a 90 cm. Entretanto, estes valores foram utilizados para identificar o fenótipo HTGW apenas em indivíduos do sexo masculino (LEMIEUX et al., 2000). Somente em 2007, houve a adoção de valores de circunferência abdominal maiores ou iguais a 85 cm em conjunto com níveis de triglicerídios maiores ou iguais a 177 mg/dL, para a definição de HTGW em mulheres (ST-PIERRE et al., 2007). Porém, mesmo após a criação destes pontos de corte para a identificação de HTGW na população feminina, a maior prevalência deste fenótipo tem sido mesmo encontrada em homens. Aproximadamente 60% a 70% dos indivíduos com este fenótipo são do sexo masculino (ST-PIERRE et al., 2007; YU et al., 2010). Desde o estudo de Lemieux e colaboradores, em 2000, diversos estudos têm comprovado que HTGW pode ser utilizado como marcador da tríade metabólica aterogênica "hiperinsulinemia, hiperapolipoproteinemia B e LDL pequenas e densas" em homens e que isto os coloca como uma população de alto risco cardiovascular e de alto risco para diabetes mellitus tipo 2 e outras anormalidades metabólicas (**Figura 3**) (LEMIEUX et al., 2000; BLACKBURN et al., 2003; SOLATI et al., 2004; ST-PIERRE et al., 2007; OKOSUM e BOLTRI, 2008; BLACKBURN et al., 2009; GRAAF et al., 2010; YU et al., 2010).

Figura 3. Fenótipo Cintura Hipertrigliceridêmica e a Tríade Aterogênica.



Fonte: ARAÚJO, Tiago Ferreira da Silva (2015). Cab – circunferência abdominal; TG – triglicerídios.

O Estudo Cardiovascular de Quebec tem encontrado que homens com obesidade abdominal, mesmo na ausência de clássicos fatores de risco para doenças cardiovasculares, como diabetes mellitus tipo 2, hipertensão e hipercolesterolemia, mas portando um perfil lipídico relacionado à hipertrigliceridemia, possuem um risco maior que vinte vezes, em comparação a homens não obesos, de apresentarem doenças coronarianas ao longo de um período de apenas cinco anos (LAMARCHE et al., 1999; SCARSELLA e DESPRÉS, 2003).

Assim, precisa-se destinar muita atenção à problemática hipertrigliceridemia e obesidade abdominal na população masculina e, principalmente, em homens provenientes de regiões consideradas em maior risco de se tornarem as futuras e grandes contribuidoras para o maior número de morte por doenças cardiovasculares, como é o caso dos países em desenvolvimento e que passam ainda por um processo de transição nutricional. Um exemplo disto é o que vem ocorrendo no Brasil. Este país atravessa um período de transição epidemiológica, com aumento do número de doenças crônicas não transmissíveis (DCNTs). A população brasileira tem apresentado mudanças de seus hábitos alimentares e

onde antes havia somente subnutrição, há, atualmente, obesidade convivendo em conjunto, às vezes, no interior de uma mesma residência (BATISTA FILHO e RISSIN, 2003; PINHEIRO; FREITAS; CORSO, 2004).

A Região Nordeste do Brasil é uma das duas últimas regiões a aderirem ao processo de transição nutricional neste país. Estudos têm encontrado altas taxas de prevalência para hipertrigliceridemia nesta região, de 35% a aproximadamente 60%, assim como para obesidade do tipo abdominal, de cerca de 20% a 60%, com as maiores taxas tendo sido encontradas, respectivamente, na Bahia e na Paraíba (BATISTA FILHO e RISSIN, 2003; PINHEIRO; FREITAS; CORSO, 2004; OLIVEIRA; SOUZA; LIMA, 2006; PONTES e SOUZA, 2009).

Em um estudo sobre a análise e comparação do perfil lipídico em homens no Estado de Pernambuco, verificou-se que mais de um terço da população masculina avaliada apresentava hipertrigliceridemia, fosse como uma dislipidemia isolada ou associada principalmente a baixos níveis de HDL-c (SANTOS et al., 2009).

No Brasil, houve um aumento de 100% na prevalência de obesidade entre os homens, ao se comparar números mais atuais com números obtidos do Estudo Nacional da Despesa Familiar (ENDEF) de 1974/1975 (PINHEIRO; FREITAS; CORSO, 2004). No Brasil, têm sido encontradas também maiores taxas de mortalidade por doenças cerebrovasculares, por exemplo, em homens do que em mulheres, independente de o acidente vascular encefálico ter sido isquêmico, hemorrágico ou ainda não definido, durante os anos de 1997 e 2003 (LOTUFO e BENSENOR, 2004).

Associando-se esses dados ao fato bem conhecido de que os homens utilizam, em uma frequência muito menor, os serviços de saúde, no Brasil, além do fato de que, quando os utilizam, os indivíduos do sexo masculino apresentam doenças comparativamente mais severas e de maior letalidade do que as mulheres, ressalta-se a necessidade de estudos voltados para a avaliação da saúde da população masculina (TRAVASSOS et al., 2002).

Duas das principais causas de aposentadoria precoce, ou seja, de redução da vida útil de homens no Brasil são a sinovite e a tendosinovite , as quais podem ser resultantes de hiperuricemia ou gota, distúrbios metabólicos que, conforme já mencionado tiveram sua presença identificada em um individuo obeso desde Morgagni, no fim do século XVIII, e desde o século XIX já se era sugerida a existência de uma relação entre hiperuricemia e

doenças cardiovasculares. Entretanto, mais de duzentos anos depois ainda se tem muito pouco estudo sobre hiperuricemia no Brasil (MOHAMED, 1879; ENZI et al., 2003; BRANCO e OLIVEIRA, 2006; GAGLIARD; MINAME; SANTOS, 2009).

A hiperuricemia é considerada um distúrbio metabólico cada vez mais freqüente nas populações (BAKER E SCHUMACHER, 2010). Uma maior prevalência de hiperuricemia tem sido verificada também em indivíduos do sexo masculino, pois homens apresentam este distúrbio cerca de quatro a cinco vezes mais que as mulheres (LI-YING et al., 2007; CONEN et al., 2004). No Brasil, um estudo realizado em um dos principais Estados da Região Sudeste, demonstrou uma prevalência de hiperuricemia de 25% (SANTOS et al., 2007). Entretanto, dados sobre hiperuricemia na população da Região Nordeste ainda são praticamente inexistentes e é inédita ainda a sua investigação em homens em associação à obesidade abdominal, a hipertrigliceridemia e ao fenótipo HTGW.

Níveis sanguíneos de ácido úrico têm sido associados ao acúmulo de gordura visceral. Entretanto, a relação entre produção de ácido úrico e síntese de triglicerídios, no fígado, baseada no acúmulo de gordura visceral ainda não foi elucida e estudos posteriores tornam-se necessários. É sugerido que a obesidade ocasione prejuízos à depuração renal de ácido úrico, reduzindo-a, e que altas concentrações de triglicerídios e de ácidos graxos relacionadas ao acúmulo de gordura visceral possam estar ligadas a um aumento na síntese *de novo* das purinas no fígado, através da via pentose fosfato, o que pode acelerar a produção de ácido úrico. Contudo é possível que haja outras explicações fisiopatológicas (MATSUURA et al., 1998; TAMBA et al., 2008).

Dietas ricas em purinas, tais como carne vermelha, frutos do mar, vegetais ricos em purina e proteína animal também têm sido associadas à hiperuricemia. A dieta americana esta pautada no consumo de carne vermelha e as regiões em transição nutricional tem aderido a este padrão nutricional. Dados do Instituto Brasileiro de Geografia e Estatística (IBGE), especificamente sobre a Pesquisa de Orçamento Familiar (POF) demonstra um aumento significativo no consumo de carne bovina no Brasil, comparando-se as médias de consumo registradas nos anos de 1987 e 1996. Esta mesma pesquisa demonstra também uma drástica redução no consumo de hortaliças. Na POF do ano de 2003, famílias com renda igual ou superior a cinco salários reportaram um volume de aquisição de carne bovina acima da média (IBGE, 1987; IBGE, 1996; BLEIL, 1998; IBGE, 2004; CHOI et

al., 2004) . Fumo e álcool também são possíveis fatores predisponentes à hiperuricemia e muito frequentes no sexo masculino (YAMAMOTO et al., 2005; MAXWELL E BRUINSMA, 2001).

Em um modelo de regressão logística multivariada, níveis de ácido úrico foram correlacionados positivamente com obesidade abdominal, triglicerídios, pressão arterial sistólica e diastólica, ingesta de álcool, níveis plasmáticos de insulina e negativamente com idade e atividade física. Outros estudos têm demonstrado que, em vez de uma associação negativa com idade, os níveis de ácido úrico no sangue aumentam ao longo dos anos, principalmente acima de 65 anos (LEE et al., 1995; BONORA et al, 1996; WALLACE et al., 2004).

Porém, diversos estudos têm levantado a hipótese de que ácido úrico em grandes concentrações sanguíneas não é apenas uma consequência, mas também um distúrbio metabólico que pode levar a sérias patologias, tais como diabetes mellitus tipo 2, doenças cardiovasculares, hipertensão arterial, doenças hepáticas e falência renal. Há, inclusive, propostas de vias mecanísticas de que hiperuricemia é fator causal e não consequência de resistência à insulina, conforme reportado anteriormente. O aumento da incidência da obesidade tem coincidido com aumento da ingestão de frutose e este fato também tem sido um problema observado em regiões em transição nutricional. Consumo de frutose ou de alimentos contendo este carboidrato tem sido ligado à hiperuricemia, levando ao desenvolvimento de diabetes. É proposto também que ácido úrico reduz os níveis de óxido nítrico (ON) endotelial, uma molécula mediadora da ação da insulina. ON aumenta o fluxo sanguíneo para as fibras musculares esqueléticas e facilita a captação de glicose, então, se houver uma diminuição nos níveis de ON endotelial, os indivíduos se tornam propensos à resistência à insulina, diabetes e outras anormalidades metabólicas (NAKAGAWA et al., 2005; JOHNSON et al., 2003; BHOLE et al., 2010). Associado à disfunção endotelial promovida pela redução de ON, o ácido úrico pode promover ainda outras alterações que culminem em HA, como por exemplo, proliferação de células musculares nos vasos sanguíneos, além da própria injúria renal (JOHNSON et al., 2003).

O ácido úrico também pode se depositar nas artérias coronárias, promover estresse oxidativo e inflamação local, alterações estas que, associadas a obesidade abdominal e às dislipidemias relacionadas com a hipertrigliceridemia, podem culminar no processo aterosclerótico (KAYA et al., 2010). Atualmente, estudos têm demonstrado que a

hiperuricemia também podem ser considerada um importante preditor de mortalidade para indivíduos com Doença Arterial Coronariana (DAC), incluindo Infarto Agudo do Miocárdio (IAM) e Insuficiência Cardíaca Congestiva (ICC) (HYUN et al., 2007), as quais pertencem ao grupo das DCNTs, que representam a atual principal causa de morte na população brasileira (MINISTÉRIO DA SAÚDE, 2010).

Assim, a hiperuricemia não pode ocasionar apenas gota, uma doença inflamatória causada pela deposição de cristais de ácido úrico nas articulações e a forma mais comum de artrite inflamatória em indivíduos do sexo masculino com o desenvolvimento de tofos, mas também diversos distúrbios metabólicos, que podem vir a diminuir a vida produtiva de seus portadores e, apesar de ser uma das doenças mais antigas a terem sido descritas, na Grécia, por Hipócrates de Cós, pouco se sabe sobre as vias mecanísticas de seu aparecimento em conjunto com outras anormalidades metabólicas, como a obesidade abdominal, a hipertrigliceridemia e o fenótipo associado cintura hipertrigliceridêmica (TOMITA et al, 2000; CHOI et al., 2004; CHOI et al., 2005; NUKI e SIMKIN, 2006).

2.5 Atividades Biológicas de Plantas

A população, em geral, faz uso de plantas medicinais no tratamento de suas doenças, mas nem sempre existem trabalhos científicos que garantam o seu uso seguro ou que comprovem realmente a sua eficácia. Devido a isto, é importante se pesquisar as atividades biológicas que essas plantas possam apresentar. Inicialmente, por meio de extratos de partes dessas plantas, procurando os melhores tipos que possam trazer os melhores compostos com melhores resultados, até que esses compostos venham a ser isolados e purificados e, por fim, possam vir a se tornar agentes terapêuticos. Dessa forma, atividades biológicas consideradas indispensáveis têm sido investigadas, tais como atividade antioxidante, antinociceptiva, anti-inflamatória, anti-diabética, anti-bacteriana, antitumoral e anti-hiperuricêmica por estarem intimamente associadas às doenças de maior prevalência e responsáveis pelas maiores taxas de mortalidade no mundo e no Brasil (MORAIS et al, 2005; HALDAR et al., 2012).

As plantas são consideradas uma abundante fonte de substâncias orgânicas que cooperam para o fornecimento de compostos secundários, dos quais algumas classes são

utilizadas na produção de produtos pelas indústrias farmacêuticas, alimentícias, dentre outras (PINTO et al., 2002). Dessa forma, muitos dos metabólitos têm sido usados no desenvolvimento de medicamentos de origem sintética, tais como a procaína, que é um anestésico local, e a cloroquina, um derivado quinolínico usado no tratamento da malária; bem como têm sido comercializados como fitofármacos, como, por exemplo: a morfina, que é um potente analgésico extraído de *Papaver somniferum*; o ácido salicílico, que é o princípio ativo da *Salix alba*, com ações antipirética, analgésica, esfoliante, anti-inflamatória e antimicrobiana; a vincristina e vimblastina, provindas de *Catharanthus roseus*, que são alcalóides anticancerígenos; a digoxina, um glicosídeo de *Digitalis* sp., muito utilizado no tratamentos de problemas cardíacos; e o taxol, um diterpeno anti-cancerígeno, derivado de *Taxus brevifolia*; dentre outros (Figura 6) (HENRIQUES et al., 1999; RATES., 2001; GANGREIRO et al., 2008).

Diversos fármacos relevantes poderiam ser ressaltados neste contexto destacando um papel importante na ciência que amplia o uso de plantas como fontes promissoras de matérias primas para uso terapêutico. Nestas buscas intensivas por novos medicamentos, os testes de triagem vêm assumindo um papel fundamental na identificação de compostos que são empregados em testes biológicos a fim de predizer qual metabólito químico é responsável por tal ação ou se possui efeito sinérgico com outros componentes, garantindo a utilização destes, na construção de futuros fármacos promissores em um curto espaço de tempo (CECHINEL FILHO & YUNES., 2001, RATES., 2001).

2.5.1 Aspectos Botânicos e Uso na Medicina Popular de *Myrciaria cauliflora*

Myrciaria cauliflora (Mart.) O. Berg (sinônimo *Plinia cauliflora*) é uma planta nativa do Brasil, pertencente à Família Myrtaceae, amplamente distribuída por todo o país, desde o Pará ao Rio Grande do Sul, sendo encontrada principalmente nas regiões de Mata Atlântica e de Cerrado. Consiste em uma pequena árvore, de 3 m a 6 m de altura (**Figura 4A**), conhecida popularmente como jabuticabeira ou simplesmente por jabuticaba, nome dado também ao seu fruto. Suas folhas apresentam de 2 cm a 6 cm de tamanho (**Figura 4B**) (IDEMIR et al., 2005; LEITE-LEGATTI et al., 2012; WU et al., 2012; WU, LONG &

KENNELLY, 2013; BORGES, CONCEIÇÃO & SILVEIRA, 2014; MERCALI et al., 2015).

Figura 4. Aspectos da *Myrciaria cauliflora.*

Fonte: ARAÚJO, Tiago Ferreira da Silva (2015). A – árvore; B – folhas; c – flores; D e E – frutos maduros.

Os frutos de *M. cauliflora*, as jabuticabas, também acompanham a distribuição das flores e crescem diretamente sobre os principais galhos e ramos da jabuticabeira (**Figura 4C e D**), de forma rápida, dentro de 40 a 46 dias, contendo de uma a quatro sementes. São redondos, com aproximadamente 2 cm a 4 cm de diâmetro, e, quando maduros, apresentam uma polpa esbranquiçada, gelatinosa, doce, levemente ácida e suculenta, de sabor agradável, sendo chamados de uva brasileira. Estudos têm reportado que as jabuticabas apresentam uma ampla variedade de nutrientes considerados clássicos, como, por exemplo, carboidratos, sais minerais, aminoácidos, como lisina e triptofano, e vitaminas B1, B2 e C, que podem ter um grande impacto na saúde humana. Especialmente, os frutos de *M. cauliflora* representam uma boa fonte de cálcio, ferro, potássio e fósforo. Essas

propriedades fazem com que possam ser consumidos, tanto na sua forma fresca, *in natura*, como na forma de sucos, geleias, sorvetes, vinagres, licores e vinhos, apresentando um grande potencial na indústria alimentícia e despertando um grande interesse econômico em várias regiões do Brasil (IDEMIR et al., 2005; LEITE-LEGATTI et al., 2012; WU et al., 2012; COSTA et al., 2013; WU, LONG & KENNELLY, 2013; MARIANNI et al., 2014).

A casca da jabuticaba também apresenta um grande potencial na indústria alimentícia, mas não em relação ao seu consumo *in natura*, pois o seu sabor é adstringente. No Brasil, tem sido considerada como uma fonte potencial de pigmentos naturais, dentre os quais se pode mencionar as antocianinas, que são compostos coloridos que podem ser potenciais substitutos para os corantes alimentares sintéticos. Os compostos fenólicos são tradicionalmente extraídos das plantas por métodos convencionais, tais como extração sólida-líquida, usando grandes quantidades de solventes orgânicos, como metanol, etanol e acetona ou soluções aquosas contendo uma pequena quantidade de ácido para manter um baixo pH, haja vista os compostos fenólicos serem particularmente instáveis (SANTOS & MEIRELES, 2011).

Os compostos fenólicos são denominados substâncias químicas que possuem, em sua estrutura, um radical hidroxila ligado a um anel benzênico. Este grupo envolve diversos componentes secundários que possuem normalmente alta polaridade e suscetibilidade a ação enzimática, tais como: taninos hidrolisados, ligninas, derivados cinâmicos, cumarinas e flavonoides. Tais compostos são provenientes da tirosina e fenilalanina do metabolismo secundário das plantas em resposta a condições de estresse (ferimentos, infecções, radiação ultravioleta, dentre outros) e podem estar na forma livre ou associados a glicosídeos e proteínas. Os compostos fenólicos nos alimentos são responsáveis por contribuir com as propriedades sensoriais (sabor, cor, odor e adstringência). Estas substâncias estão envolvidas com efeitos benéficos biológicos com propriedades anti-inflamatória, antioxidante, antialérgica, antiviral, anticolesterolêmica e antioxidante (SÁNCHEZ-MORENO., 2002; SOARES, 2002; NACZK & SHAHIDI., 2004; MALACRIDA & MOTTA., 2005).

A grande relevância, destinada nesses últimos anos aos frutos da jabuticabeira, não é derivada apenas do potencial alimentício de sua polpa ou de sua casca para a obtenção de corantes, mas também, e principalmente, do potencial biológico que as cascas podem apresentar, o qual se baseia nesse conteúdo de substâncias com propriedades medicinais que possam existir nessas cascas de jabuticaba. O epicarpo da jabuticaba é fino, frágil e,

quando o fruto se encontra amadurecido, sua coloração varia de roxa escura a praticamente preta (**Figura 4E**); e, em muitas culturas, também tem sido reconhecido que frutos de cor escura demonstram um bom potencial para melhorar a qualidade da saúde humana, trazendo muitos benefícios. Inclusive, o chá da casca de *M. cauliflora* tem sido utilizado, pela medicina tradicional, para o tratamento de asma, hemoptise, angina, disenteria, erisipela e inflamações das tonsilas palatinas (REYNERTSON et al., 2006; LEITE-LEGATTI et al., 2012; WU et al., 2012; COSTA et al., 2013; CALLONI et al., 2015).

A riqueza fenólica da *M. cauliflora* a torna em uma potente fonte de antioxidantes naturais. Compostos antioxidantes ainda têm sido associado com a redução de massa corpórea, alterações de hormônios relacionados com a obesidade, redução da resistência insulina e efeitos benéficos no diabetes mellitus tipo 2 e doença de Alzheimer. Estudos recentes, utilizando o epicarpo da *M. cauliflora* em dietas alimentares, demonstrou o potencial efeito antioxidante no plasma de ratos, que pode ser atribuído ao conteúdo de antocianinas da casca. Atuação de depsídeos e antocianidinas dos frutos também tem sido associada com a diminuição de inflamação, provocada pela exposição à fumaça de cigarros. Além disso, diversos compostos secundários identificados nesta espécie também estão relacionados com atividades biológicas já confirmadas cientificamente, como, por exemplo, a quercetina e a rutina, que possuem atividade antitrombocítica e agem inibindo a atividade das lipoxigenases, cicloxigenase, fosfolipase A2 e crescimento de fibroblastos (KOGANOV et al., 1999; HOMMAN et al., 2000; ISIHIWAJ et al., 2000; LEITE et al., 2011; PRIOR et al., 2012).

Por isso, extratos de plantas, ricos em tais compostos, apresentam um grande potencial para serem agentes terapêuticos, e, conforme os potenciais biológicos desses compostos, podem atuar na melhora do prognóstico das doenças mais prevalentes no mundo e no Brasil, responsáveis por altas taxas de mortalidade, que são as doenças crônicas não-transmissíveis, como as doenças cardiovasculares. Dor, inflamação, estresse oxidativo, níveis elevados de glicose, de colesterol, de triglicerídios, tem sido associados a essas doenças, ou como fatores causais ou como consequências, que agravam e dificultam o quadro de melhora do indivíduo. Resposta inflamatória exacerbada, por exemplo, com elevação de citocinas de fase aguda, estão intimamente associadas com o quadro de obesidade e de resistência à insulina, propiciando o aparecimento de diabetes mellitus e aumentando as chances de o indivíduo vir a ter doenças cardiovasculares, ampliada pelo aumento do aporte hepático de ácidos graxos livres, devido a uma maior liberação para a

corrente sanguínea por intermédio da elevação dessas citocinas, provocando o aparecimento de dislipidemias, como elevação dos níveis de triglicerídios e de colesterol, e diminuição de HDL-colesterol. Dessa forma, é muito importante que os novos agentes fitoterápicos possuam essas atividades biológicas (REAVEN., 1988; GRUNDY., 2006; ECKEL; GRUNDY; ZIMMET, 2005; LI & FORD., 2006; JOHNSON & WEINSTOCK., 2006; HALDAR et al., 2012; BORGES; CONCEIÇÃO; SILVEIRA, 2014).

2.5.2 Aspectos Botânicos e Uso na Medicina Popular de *Crataeva tapia*

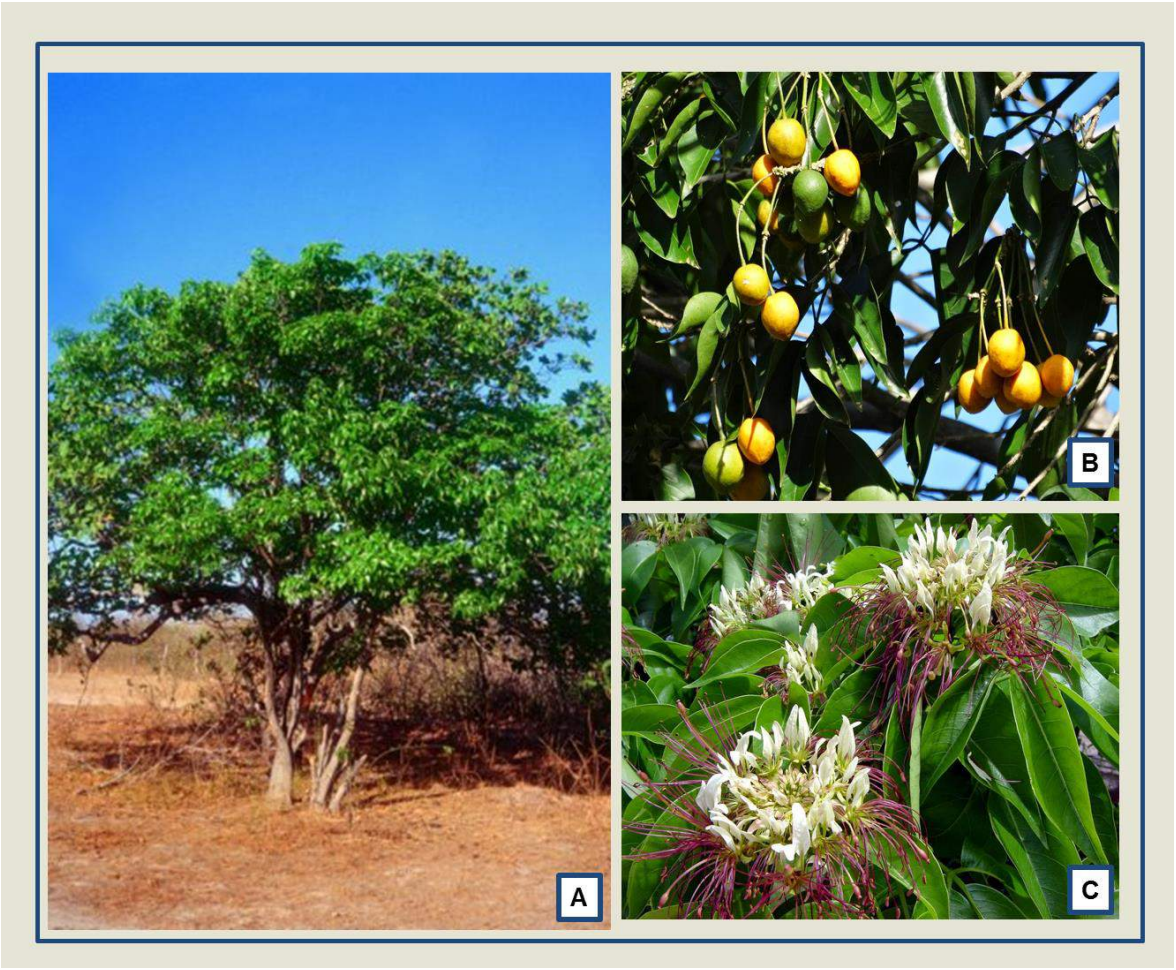
Crataeva tapia é uma planta pertencente a família Caparidaceae que cresce comumente em Florestas Tropicais Atlânticas e no Pantanal. *C. tapia* é conhecida popularmente no Brasil como "Trapiá" ou "Paudalho". Sua árvore (**Figura 5A**) pode apresentar de 2 a 25 metros de altura e é dotada de copa arredondada e densa que pode atingir até 20 metros de diâmetro, por isso trata-se de uma espécie muito recomendada para a arborização e recomposição de áreas degradadas (PATRISSOLI et al., 2007).

Os frutos (**Figura 5B**) são comestíveis e em sua maior parte consumidos pela população na forma de refresco ou de bebida vinosa (LORENZI et al., 2002). As flores (**Figura 5C**) são vistosas e nascem no ápice dos ramos, reunidas em cachos corimbiformes (cacho em que as floras saem de pontos diferentes da mesma haste). Nos ramos, as flores apresentam aproximadamente 7 cm de diâmetro. Na medicina popular a casca do tronco de *C tapia* é usada como tônico, estomáquico, antidisentérico, febrífugo e no tratamento do diabetes (ALVES et al., 2012).

Estudo prévios demonstraram que o extrato aquoso obtido da casca da árvore de *C tapia* apresentou atividade hemaglutinante, estando esta atividade associada a presença de lectinas. As lectinas são proteínas versáteis que tem capacidade de ligar-se a carboidratos e a capacidade de interação entre as lectinas e os carboidratos em diferentes superfícies celulares pode promover algumas atividades biológicas a este tipo de proteínas (PAIVA et al., 2003; FRAGUAS et al., 2004; ANIULITE et al., 2006; SILVA et al., 2011). Diversas lectinas purificadas de plantas tem exercido importantes atividades biológicas dentre elas: antitumoral (ARAÚJO et al., 2011), anti-inflamatória (MELO et al., 2010), antimicrobiana (SIMÕES et al., 2012), analgésica (ARAÚJO, et al., 2011), antioxidante (SANTOS et al.,

2013), inseticida (ARAÚJO et al., 2012), anticoagulante (ARAÚJO et al., 2011) e hipoglicemiante (KAVALALI et al., 2003).

Figura 5. Aspectos da *Crataeva tapia*.



Fonte: belezadacaatinga.blogspot.com. A – árvore; B- frutos; C – flores.

A propriedade hipoglicemiante das lectinas observada em estudos tem sugerido o potencial terapêutico destas moléculas no tratamento do diabetes. Estudos com lectinas extraídas do feijão de soja demonstraram que esta lectina foi capaz de diminuir 17,3% os níveis glicêmicos de animais diabéticos (HEMALATHA et al., 2011). Lectinas extraídas de *Agaricus bisporus* também foram hábeis no tratamento da hiperglicemia em animais diabéticos (WANG et al., 2012). Desta forma, as lectinas tem despertado o interesse para o

tratamento do diabetes, inclusive os estudos tem associado o efeito hipoglicemiante destas proteínas a propriedade que elas teriam em estimular o crescimento das células beta pancreáticas produtoras de insulina. Assim, o aumento dos níveis de insulina poderiam regularizar os níveis sanguíneos de glicose (HEMALATHA et al., 2011; WANG et al., 2012).

2.5.3 Aspectos Botânicos e Uso na Medicina Popular de *Indigofera suffruticosa*

A *Indigofera suffruticosa* é uma espécie originária da Antilha e América Central (ALMEIDA, 1993) mais predominante por toda a América Tropical. No Brasil, encontra-se distribuídas em alguns estados: Mato Grosso (FERNANDES, 1987), Alagoas (RIBEIRO, 1984), Paraíba (RIET-CORREA, 2000), Ceará, Rio Grande do Norte, Pará e Pernambuco (NETO et al., 2001). É descrita como uma planta arbustiva (**Figura 6A**), medindo 1m a 2 m de altura, possuindo ramos pubescentes, caule anguloso, de cor acizentada, folhas pinadas (**Figura 6B**) compostas por 7 a 15 folíolos oblongos ou ovais, glabros na face e no verso, apresentando flores pequenas (**Figura 6C**), numerosas, roseas ou amareladas, em racemos axilares, e seu fruto é uma pequena vagem falciforme com 6 a 10 sementes medindo 25 mm de comprimento (BRAGA, 1976).

A *I. suffruticosa* pode ser relacionada a outros nomes populares, tais como, jiquilite, tzitzupu, anil do campos, anileira-da-índia, anileira verdadeira, caá-chica, caá-chira, timbó-mrim, timbozinho, e indigueira. As primeiras investigações dos componentes químicos de *I. suffruticosa* foram realizados por Miller e Smith, 1973, utilizando extrato de sementes. Esta espécie apresenta na população tais propriedades fitoterápicas: antiespasmódicas, sedativas, diuréticas, purgativas, odontálgicas (LORENZI, 1982; BRAGA, 1985).

Estudos farmacológicos da *I. suffruticosa*, destacam as aplicações clinicas utilizando partes aéreas e de folhas com as seguintes atividades: citotóxica para células embrionárias em ratos (LEITE et al., 2004), antimicrobiana contra a bactéria gram-positiva *Staphylococcus aureus* (LEITE et al., 2006), antifúngica contra dermatófitos *Microsporium canis e Trichophyton rubrum* (LEITE et al., 2006), anti- tumoral (VIEIRA et al., 2007), e antiinflamatória na redução de edema de pata de camundongos (LEITE et al., 2003).

Figura 6. Aspectos da *Indigofera suffruticosa*.

Fonte: Silva, 2010. A – Planta; B – Ramos com folhas e inflorescências; C – Ramos floridos.

Desta forma, os compostos naturais possuem um papel altamente significativo no desenvolvimento de novas drogas com perfil farmacológico. Isto é particularmente evidente no caso de diversas doenças (NEWMAN et al., 2003). Mesmo assim a produtividade em relação à identificação de compostos naturais efetivamente úteis como ferramentas de pesquisas biológicas ou como candidatos a protótipos de fármacos e outras substâncias de interesse é relativamente baixa, sugerindo que novas pesquisas sejam realizadas para investigar e avaliar tais compostos que possam atuar de forma mais específica e seletiva, com menos efeitos adversos. Além disso, a OMS tem recomendado a elaboração de políticas nacionais voltadas à integração e inserção da Medicina Tradicional

(MT) e da Medicina Complementar Alternativa (MCA) aos sistemas oficiais de saúde, com foco na Atenção Primária à Saúde (APS).

As plantas medicinais e seus derivados estão entre os principais recursos terapêuticos sendo utilizados há muito tempo pela população brasileira nos seus cuidados com a saúde. Pelo SUS (Sistema Único de Saúde), até o ano de 2010, apenas dois fitoterápicos eram oferecidos como medicamentos, aqueles produzidos com guaco e espinheira santa. A partir de então, a rede pública passou a contar com mais seis produtos, sendo eles: fitoterápicos formulados com alcachofra, aroeira, cáscara sagrada, garra do diabo, isoflavona da soja e unha de gato. Em 2012, o Ministério da Saúde publicou a Portaria MS/GM nº 533, na qual é estabelecido o elenco de medicamentos e insumos da Relação Nacional de Medicamentos Essenciais – RENAME, onde passaram a ser contemplados mais medicamentos fitoterápicos.

As ações para implementação dessas políticas nacionais buscam ampliar a oferta de serviços e produtos relacionados à fitoterapia no SUS de forma segura e racional e como forma de ampliar as opções terapêuticas, estados e municípios passaram a ofertar serviços de fitoterapia em sua rede, aprovaram políticas e legislação específicas, instalaram hortos e laboratórios de produção com a finalidade de disponibilizar plantas medicinais e seus derivados, prioritariamente, na atenção básica, além do fornecimento de publicações para profissionais de saúde e população, sobre uso racional desses produtos.

Deste modo, com base em estudos científicos que relatam os potenciais farmacológicos de determinados compostos extraídos de plantas justifica-se a necessidade de extrair e avaliar tais compostos na casca do fruto maduro de *M. cauliflora*, na folha da *I. suffrutica* e na entrecasca de *Crataeva tapia* que possam contribuir com o arsenal terapêutico. *Estas espécies* demonstram poder apresentar um grande potencial biotecnológico. Mas apesar de ter sido despertada recentemente a atenção para estudos que investiguem os seus potenciais farmacológicos, estudos com compostos extraídos da casca de fruto da jabuticaba ainda são praticamente inexistentes, pois infelizmente *M. cauliflora* ainda é uma espécie subaproveitada da flora brasileira. Desse modo, o presente estudo propõe uma investigação do potencial terapêutico de extratos obtidos da casca do fruto maduro de *M. cauliflora, Crataeva tapia e Indigofera sufrruticosa* em diferentes modelos experimentais que são um dos principais distúrbios contribuintes para o aumento do número de óbitos provocados por doenças crônicas não transmissíveis e por infecções no Brasil e no mundo.

III. OBJETIVOS

3.1 Objetivo Geral

Investigar a relação entre a hiperuricemia, a presença de distúrbios metabólicos e as atividades biológicas de *Myrciaria cauliflora*, *Crataeva tapia* e *Indigofera suffruticosa*.

3.2 Objetivos Específicos

- Avaliar a relação entre obesidade abdominal, hipertrigliceridemia (ambos de forma isolada e em associação, constituindo o fenótipo cintura hipertrigliceridêmica), e a presença de hiperuricemia na população masculina não-diabética do Nordeste brasileiro;
- Investigar a relação entre hiperuricemia e o risco cardiovascular na população masculina não-diabética do Nordeste brasileiro;
- Obter extratos orgânicos fracionados (fração éter, fração acetona e fração metanol) oriundos do epicarpo do fruto maduro de *Myrciaria cauliflora*;
- Realizar a identificação das principais classes de compostos em extratos fracionados de *M. cauliflora*;
- Avaliar a toxicidade oral aguda dos extratos fracionados de *M. cauliflora*;
- Investigar o potencial anti-diabético e anti-hiperuricêmico da fração acetônica do epicarpo maduro de *M. cauliflora*, acessando o potencial biológico desse extrato na reversão das alterações bioquímicas e histológicas promovidas por esses distúrbios metabólicos;
- Avaliar as atividades antitumorais, anti-inflamatória, antinociceptiva e antioxidante dos extratos fracionados do epicarpo maduro de *M. cauliflora*;
- Investigar o efeito da atividade anti-diabética da lectina extraída da casca de *Crataeva tapia* (CrataBL);

- Avaliar o efeito antibacteriano de extratos orgânicos fracionados obtidos das folhas de *Indigofera suffruticosa*.

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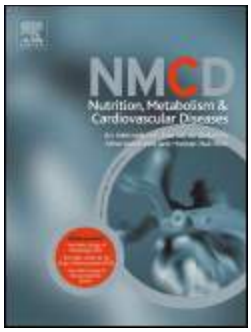
V. ARTIGO 1

"Hypertriglyceridemic Waist and Hyperuricemia: A Tool for Cardiovascular Risk in Non-Diabetics Brazilian Men"



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Hypertriglyceridemic Waist and Hyperuricemia: A Tool for Cardiovascular Risk in Non-Diabetics Brazilian Men

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ABSTRACT

Background and Aims: Abdominal obesity (AO) with hypertriglyceridemia [hypertrigliceridemic waist – HTGW phenotype] has been associated to an atherogenic triad, which it consists in an increase of plasma levels of insulin, apolipoprotein B and small dense low-density lipoprotein particles. The aim of this study was to investigate the relationship between HTGW and hyperuricemia among 3,620 non-diabetic Brazilian men. **Methods and Results:** HTGW was defined as Waist Circumference (WC) ≥ 90cm and Triglycerides (TG) ≥ 2.0mmol/L. Hyperuricemia was considered when values of uric acid≥0.416 mmol/L. Receiver operating characteristics curve analysis stablished the best WC and TG cut-off points considering the incidence of hyperuricemia. The relationship between HTGW and hyperuricemia was estimated using odds ratio from the logistic regression model. The prevalence of HTGW and hyperuricemia was 22.1% and 11.1%, respectively. AO hypertriglyceridemia only showed influence on hyperuricemia (OR = 3.7 and 2.8, respectively). However, there was no difference between the isolated effects of AO and hypertriglyceridemia on hyperuricemia, checked by similarity of ROC curves (AUC = 0.715 and 0.719, respectively, p = 0.9555). The average uric acid did not differ among individuals with these abnormalities. But, despite of AO and hypertriglyceridemia similar and independent effects, when HTGW was present, the association with hyperuricemia was considerably higher (OR = 4.3), and especially after the adoption of more specific and sensitive cutoff points of 91cm, for WC, and of 1.73 mmol/L to TG. Hyperuricemia also had a significant relationship with high cardiovascular risk (OR = 3.5), positively correlated with AO and hypertriglyceridemia. **Conclusion:** AO, hypertriglyceridemia and HTGW was correlated with hyperuricemia and the relationship between hypertriglyceridemia and hyperuricemia appeared to be the main factor for the contribution of HTGW. Hyperuricemia also been highly associated with high cardiovascular risk in this study.

Key words: Hypertrigliceridemic Waist Phenotype, Hyperuricemia, Cardiovascular Risk, Brazilian Men.

INTRODUCTION

Obesity is recognized worldwide as an important nutritional disorder, affecting developed countries, but also countries still in development.[1] The high and increasing prevalence of obesity and its comorbidities have represented a serious challenge for public health systems and medical care in all countries in which this disorder is present.[2]

Brazil has the most populous nation in Latin America, representing one of the greatest populations in the world.[1-4] In 1989, population studies showed a significant regional difference in the prevalence of overweight and obesity with a low prevalence in the Northeast and a high prevalence in the Southeast region of Brazil.[2]

However, this country has experienced a rapid economic expansion, and the Northeast region has undergone certain changes in feeding patterns in the socio-economic life, and other habits, defined as nutritional transition, and the adoption of these lifestyle of life has significantly contributed to the increased prevalence of obesity in this region Brazilian.[1,3,4]

Obesity increases the risk of Chronic Noncommunicable Diseases (CNDs), which are responsible for 72% of deaths in the Brazilian population.[5] Obese individuals are more likely to develop diabetes mellitus and cardiovascular and atherosclerotic diseases (DCVAs), which represent 4.6% and 29.4% mortality in Brazil, respectively.[6] The likelihood of developing diabetes mellitus DCVAs and is even higher in patients with abdominal obesity type, common in males.[7,8] Abdominal adipose tissue is more metabolically active and may promote hypertriglyceridemia, dyslipidemia characterized by one hypertriglyceridemia (HTG), which in turn can cause other lipid abnormalities, such as changes in the chemical composition of the LDL particles, assisting in the development of small dense LDL. Hypertriglyceridemia may also have a positive correlation with the development of Cardiovascular Diseases (CVDs).[9,13]

Population studies involving the Northeast region of Brazil showed high prevalence of hypertriglyceridemia, ranging from 35% to approximately 60%, with the highest rates in the states of Bahia and Paraiba.[10,11] Previously was identified in

Pernambuco, another important state in Brazil's Northeast region, that hypertriglyceridemia is present in more than one third of the male population, as an isolated dyslipidemia or associated mainly to low HDL-cholesterol levels.[12]

When there is the concomitant presence of abdominal obesity and hypertriglyceridemia in the same individual, there is the presence of a phenotype called Waist hypertriglyceridemic (HTGW). This phenotype was described by Lemieux and cols. (2000) as an efficient and low-cost marker to identify patients at high cardiovascular risk. It is believed that these individuals are characterized by the presence of a metabolic triad with hyperinsulinemia, elevated serum levels of apolipoprotein B particles and small dense LDL.[9] Since then, HTGW phenotype has been shown to be an important tool for the identification of cardiovascular risk.[14-16] However, little is known about the effect of HTGW phenotype in other metabolic abnormalities, such as hyperuricemia, in which there is increased uric acid levels in the blood.

Hyperuricemia is a metabolic abnormality recognized for a long time and also very prevalent in men. People with hyperuricemia show a greater predisposition to gout, an inflammatory disease caused by deposition of uric acid in the joints.[17] Hyperuricemia may be secondary to metabolic disorders such as obesity and hypertriglyceridemia. In addition, recent studies have found that excess uric acid may also play an important role in the pathogenesis of other metabolic diseases, which are quite frequent in patients with abdominal obesity and / or hypertriglyceridemia. yperuricemia may appear in an individual as a result of these metabolic abnormalities and, in turn, may lead to other disorders as a consequence of metabolic abnormality, and in turn, can lead to other disorders, but also likely to be exacerbated by obesity disorders have caused and the hypertriglyceridemia, beyond from to be able to exacerbate already disorders caused by obesity and hypertriglyceridemia.[18] Uric acid can be deposited in the coronary arteries, promoting oxidative stress and inflammation, and contribute significantly to the development of hypertension and other CVDs.[17,19,20,21,22]

This study aimed to evaluate the relationship between HTGW phenotype and hyperuricemia, and in addition also to verify the influence of isolated abdominal obesity and isolated hypertriglyceridemia on hyperuricemia. This study also aimed to identify cutoffs of waist circumference (WC) values and serum levels of triglycerides, more

related to the presence of hyperuricemia in non-diabetics Brazilian men. This study also aimed to analyze the influence of hyperuricemia on the cardiovascular risk of these individuals.

METHODS

Population Study

This study was conducted from 2008 to 2010 and included 3,620 men aged 20 to 79 years. All were randomly selected to represent the 51,871,449 inhabitants of northeastern Brazil (IBGE, 2010). However, the total number of participants is more than 3,620 men, since individuals with cardiovascular, hepatic, renal, or endocrine, as well as diabetes mellitus were excluded from the study. Individuals treated with drugs that interfere with insulin sensitivity or in lipid levels were also excluded. This study was approved by the Ethics Committee in Research (CEP ASCES 006/08), following the recommendations of the Declaration of Helsinki and all the volunteers in this study signed an Informed Consent to express in writing their participation.

Blood Samples and Biochemical Analysis

After fasting for 12 hours blood samples were collected by vacuum venipuncture, between 7 am and 9 am hours, in two appropriate tubes: one with potassium ethylenediaminetetraacetic in combination with sodium fluoride; and another tube without anticoagulant (Beckton Dickinson, EUA), the so-called dry tube. The samples were immediately centrifuged at 2500 xg for 15 minutes at 4 ° C (Sorvall RC6, NC, EUA). Plasma was obtained from the first tube, while the serum was obtained from the dry tube.

Serum concentrations of uric acid were determined by enzymatic reaction using uricase, through Trinder method's (MERCK, GE). Hyperuricemia was defined as uric acid concentration $\geq$ 0.416 mmol/L.[23] Plasma concentrations of glucose (G) and serum

total cholesterol (TC) and triglycerides (TG) were determined by specific enzymatic methods (MERCK, GE). Serum levels of HDL-cholesterol (HDL-c) were determined enzymatically after precipitation with phosphotungstic acid and magnesium chloride (MERCK, GE). The levels of LDL-cholesterol (LDL-c) and VLDL cholesterol (VLDL-C) were obtained by Friedewald equation: LDL-c = TC – HDL-c – TG/5; onde VLDL-c = TG/5.[12] Insulinemia was determined by Microparticle Enzyme Immunoassay (MEIA) (Abbott Laboratories, GE), with cross-reactivity to human pro-insulin of only 0.016%. Insulin resistance (IR) was evaluated by Homeostatic Model Assessment - IR (HOMA-IR), through the equation: [fasting insulinemia (μU/mL) x fasting glycemia (mmol/L)] / 22.5.[24]

Anthropometric Measurements, Blood Pressure and Lifestyle

WC (cm) was determined in the middle of the axillary line, between the bottom edge of the last rib and the iliac crest. The height and the body weight of the subjects was also determined. Each subject had blood pressure was measured three times using a sphygmomanometer, after standing at least 20 minutes. The systolic (SBP) and diastolic (DBP) were determined as the average between the second and third measurements. Data on the lifestyle of individuals were obtained by questionnaire, by which it was possible to access the habits related to smoking, physical inactivity and alcohol consumption in this male population. The presence of alcohol consumption was defined as the participants reported having consumed at least 12 drinks in a year, and cigarette smoking was identified when the participants reported that they had smoked at least 100 cigarettes over a lifetime. Sedentary people were defined as those who reported no physical exercise for thirty minutes at least three times a week.

Abdominal Obesity, Hypertriglyceridemia and HTGW Phenotype

The cutoff points ≥90 cm, WC, and ≥ 2.0 mmol/L of TG levels were used to identify the presence, respectively, AO and HTG. The HTGW phenotype was defined

when AO and HTG disorders were present simultaneously in the same individual.[9] WC values cutoffs and TG specific to the study population were also identified.

Framingham Risk Score

The Framingham Risk Score (FRS) was used to estimate the absolute risk of acute myocardial infarction (MI) and death in 10 years. For each individual was assigned a score based on age, serum levels of TC and HDL-C, smoking and blood pressure. Then, the score was converted into absolute risk.[25]

Statistical Analysis

Data were expressed as mean ± standard error of the mean and frequency. The analysis of Receiver Operating Characteristic (ROC) was used to find the best cutoff points of the circumference of the waist triglyceride levels as predictors of hyperuricemia.

Logistic regression was used to assess the relationship between AO isolated, isolated HTG, HTGW phenotype and hyperuricemia, according to the cutoff points previously established and the cutoff points found in this study. The Pearson correlation test was used to evaluate the possible correlation between uric acid values, WC and TG levels in patients at high risk of MI and death in 10 years. All statistical analyzes were performed using Statview (version 5.0, 1998) and MedCalc (version 11.3.0, 2010). The level of significance was set at $p < 0.05$.

RESULTS

Table 1 shows the general characteristics of the study population: age, abdominal circumference, BMI, SBP, DBP, glucose, insulin, HOMA-IR, TC, LDL-C, VLDL-C,

HDL-C, TG and uric acid. In **Table 1**, there are also showed the characteristics related to lifestyle habits of non-diabetic Brazilian men, demonstrating that 19.8% are smokers and 49.2% are sedentary. Alcoholism was present in more than half of the individuals in the sample of this study.

The prevalence found in this population of AO and HTG were, respectively, 57.4% (95% CI = 55.0% - 60.0%) and 27.1% (95% CI = 25.4% - 28.8%). Table 2 shows the prevalence of these two disorders when present alone or in combination constituting the HTGW phenotype, which was in a little over a fifth of the population studied.

Hyperuricemia presented a prevalence of 11.1% (CI 95% = 10.0% - 12.2%). Both AO as HTG were independent risk factors for hyperuricemia. In **Table 3**, there are the odds ratios of isolated AO, isolated HTG and HTGW phenotype for hyperuricemia. After adjusting for age, HOMA-IR, SBP, DBP, alcohol consumption, smoking, physical inactivity and TG, AO had a high and significant OR for hyperuricemia. HTG, after adjusting for the same parameters, with replacement of TG levels by WC values, showed a significant OR, but lower than the AO, for hyperuricemia. When we analyzed the influence of these metabolic disorders in a manner associated on hyeruricemia, constituting the HTGW phenotype, it was found a high and significant OR of 4.3. **Table 3** also shows values OR of AO, HTG, and of the HTGW phenotype, for hyperuricemia prevalence, when considering specifical cutoffs of WC and TG levels for these men, when considering specifical cutoffs of WC and TG levels for the population studied, which presented OR values of HTG considerably higher than those found using the standard cutoff points of 91 cm and 1.73 mmol/L.

The ROC curves constructed to obtain the best cutoff points of WC and TG levels, to identify hyperuricemia in these non-diabetics brazilian men, presented values of Area Under Curve (AUC) equal to 0.715 (CI 95% = 0.643 – 0.779; p=0.0001), as shown in **Figure 1A**, and 0.719 (CI 95% = 0.647 – 0.783; p=0.0002), as shown in **Figure 1B**, respectively, with no statistical difference between the two curves (p=0.9555), as demonstrated in **Figure 1 C**. The ROC curves showed that the best cutoff point of WC for identification of hyperuricemia was equal to 91 cm, with sensitivity values equal to 85.0% (CI 95% = 62.1% to 96.8%) and specificity equal to 52.2% (CI 95% = 44.2% to 60.1%); and demonstrated that the best criterion for the serum TG levels with higher

correlation with the diagnosis of hyperuricemia was 1.73 mmol/L with a sensitivity of 75.0% (CI 95% = 50.9% to 91.3%) and specificity equal to 72.7% (CI 95% = 65.1% to 79.4%).

Figure 2 demonstrates the serum levels of uric acido, according to the presence of isolated and associated AO and HTG, constituting the HTGW phenotype, identified through the most sensitive and specific cutoff points for the population tested. Individuals with one of the disorders (AO or HTG) or the two in combination had significantly higher serum concentrations of uric acid. There was no significant difference (p = 0.3186) between uric acid values of individuals with isolated AO and isolated HTG. However, individuals with HTGW phenotype had higher serum uric acid compared to individuals with isolated abdominal obesity or isolated hypertriglyceridemia

When we evaluated the relationship between hyperuricemia and high risk of MI and death in 10 years, hyperuricemia had an OR of 3.5 (2.6 - 4.7, p <0.0001) for the frequency of high values of FRS. In individuals with high ERF (absolute values greater than 20%), serum uric acid levels showed a strong positive correlation with the levels of triglycerides (r= 0.414; CI 95% = 0.359 – 0.466; p<0.0001), as well as WC values (r= 0.328; CI 95% = 0.269 – 0.384; p<0.0001).

DISCUSSION

In this population-based study, we ascertained the association between isolated abdominal obesity, isolated hypertriglyceridemia, hypertriglyceridemic waist phenotype and the frequence of hyperuricemia in non-diabetic individuals living in Northeastern region of Brazil. In our knowledge, this is a first report for the relationship between HTGW phenotype and hyperuricemia in non-diabetics men.

AO proved to be a very present disorder in men in the Northeast of Brazil. AO was more frequent than in other countries that make up Latin America, such as Argentina, Colombia, Ecuador, Mexico and Venezuela.[26] Excess of adipose tissue n the abdominal region favors the development of diseases such as diabetes mellitus. Several

literature data suggest that changes in the regulation of many physiological and cellular functions arising from diabetes promote deregulation of control of blood pressure and lipid metabolism and purine (Bonora et al., 2008). However, the research conducted in this study excluded the participation of diabetic subjects, demonstrating that the presence of AO in more than half of the individuals may also contribute to the development of severe diabetes and other conditions that increase the risk of CVDs.

A study conducted by Batista Filho and Rissin (2003)[3] in Pernambuco, an important state in northeastern Brazil, considers that increasing rates of obesity in this region is related to certain changes in feeding patterns and lifestyle habits adopted by the population, which are characteristic of areas where nutritional transition. These authors also suggest that this region operates a rapid polarization of the obesity / dyslipidemia binomial.

Després and Lemieux (2006)[7] eported that HTG is a dyslipidemia that is closely associated with AO. HTG was also reported in non-diabetic men the Northeast in nutritional transition. The HTG frequency was more present in this study than in countries that have gone through the nutrition transition, as is the case of the United States, where it was found that 22.5% of men have this dyslipidemia.[14]

According to Lemieux et al. (2000)[9], the concomitant presence of HTG and AO characterizes, in a subject, the HTGW phenotype, which was responsible for increase by 20 times the cardiovascular risk of non-diabetic men in the Canadian population. The frequency of HTGW phenotype in non-diabetics Brazilian men was even greater than in the study conducted with the population of Canadian non-diabetic men, where HTGW phenotype was present in 12%, or in populations of other regions, as for example, in the United States and in Israel, where 13.1% and 13.0% of men had this phenotype, respectively.[14,15]

Significants OR demonstrated the association between isolated AO and HTG, and hyperuricemia in non-diabetics Braziliand men. These results are in agreement with the direct correlation observed between increased serum levels of TG or values of WC and serum uric acid levels, observed in Japanese populations.[27,28] However, in this study, when abdominal obesity and hypertriglyceridemia were associated (constituting HTGW

phenotype) there was a potentiation of pathophysiologic relationship between these disorders and hyperuricemia. Thus, the results suggest, inedited character, a direct association between the HTGW phenotype and hyperuricemia.

The mechanistic basis for the development of hyperuricemia involve mechanisms related to an increased production of uric acid and / or a decrease in renal excretion.[22] The pathophysiological relationship observed among OA, HTG, HTGW phenotype and hyperuricemia in this study was adjusted for the following variables: age, HOMA-IR, SBP, DBP, ethylism, tabagism and sedentarism; which may reduce renal excretion of uric acid and promote hyperuricemia.[22,28] Thus, we believe that the pathophysiologic relationship between hyperuricemia and metabolic disorders may be more involved with increasing uric acid biosynthesis in these individuals.

Studies have suggested that high concentrations of TG and fatty acids released by the abdominal adipose tissue can increase the *de novo* liver synthesis of purines through the pentose phosphate pathway, which in turn increases the production of uric acid in AO and HTG subjects.[27,28] Furthermore, the presence of hyperuricemia in hypertriglyceridemic individuals may be related to an increase in the activity of enzymes that are involved in the biosynthesis of this acid, such as xanthine oxidase, which can show greater activity in subjects with HTG.[30]

The pathophysiological relationship between HTGW phenotype and hyperuricemia was even stronger when cut-off points more sensitive and more specific were adopted to men found in northeastern Brazil. These cut-off points reported in the present study differed from the adopted cut-off initially by Lemiuex and cols. (2000)[9] for the determination of the HTGW phenotype. Our cut-off values were still higher than those adopted by the International Diabetes Federation (IDF) in 2005, an international contemporary guide to the diagnosis of HTG and AO. This guide recommends specific cutoff points for different ethnic groups, for example, the cutoff points of 90 cm and 1.77 mmol/L proposed, respectively, for the identification of AO and HTG to Asian populations (China and South Asia). Though, these same cut-offs are recommended by this guide to the diagnosed of AO and HTG in populations of Central and South America, including Brazilian population.[29] As Brazil has a very mixed population, we speculate that the values of cut-off proposed by the IDF could not represent a real way the ethnic

characteristics of this population. Distinctly, our study shows, in inedited character, cut-offs more appropriate (WC ≥ 91 cm and TG ≥ 1.73 mmol/L) for the determination of AO and HTG in men, and proposes a better pathophysiological relationship between these metabolic disorders and hyperuricemia in non-diabetic men in Northeast Brazil, when compared with the same disorders diagnosed using the proposed cutoff points initially by Lemiew and cols. (2000)[9].

Our study emphasizes the importance of hyperuricemia to the high risk of developing MI and death in 10 years in non-diabetic men. Our results corroborate those reported by Krishnan et al. (2006)[23], in which it was found high levels of uric acid that were able to increase the risk for this CVD in 1.26 times. Recent studies have revealed the importance of hyperuricemia in the development of CVDs that are currently responsible for the highest mortality rates, and even have been observed increases in tsunamic proportions in the global prevalence of these diseases.[17,19,21]

In conclusion, AO and HTG, alone or combined, forming the HTGW phenotype, were very frequent in this study. These metabolic disorders present an important pathophysiological relationship with the development of hyperuricemia in non-diabetic men in the Northeast of Brazil, which could even increase the risk of development of MI and death in 10 years in this population.

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Table 1. Basal characteristics of non-diabetic men from the region in nutritional transition.

Variables[a]	Values
Age (years)	44.4 ± 0.25
Waist circumference (cm)	92.6 ± 0.19
Body mass index (kg/m^2)	25.8 ± 0.07
Sistolic blood pressure (mmHg)	127.9 ± 0.38
Diastolic blood pressure (mmHg)	83.9 ± 0.22
Uric Acid (mmol/L)	0.30 ± 0.01
Triglycerides (mmol/L)	1.71 ± 0.02
Total Cholesterol (mmol/L)	4.93 ± 0.02
HDL-cholesterol (mmol/L)	0.98 ± 0.01
LDL-cholesterol (mmol/L)	3.23 ± 0.02
VLDL-cholesterol (mmol/L)	0.69 ± 0.01
Glucose (mmol/L)	4.76 ± 0.01
Insulin (µU/mL)	8.39 ± 0.17
Homeostasis model assessment of insulin resistance	1.83 ± 0.04
Ethylism	55.8
Tabagism	19.8
Sedentarism	49.2

[a] Continuous variables shown as mean ± SEM and categorical variables as %.

Table 2. Prevalence of abdominal obesity, hypertriglyceridemia and hypertriglyceridemic waist phenotype in non-diabetic men come from the region in transitional nutrition.

Study Population	N	%	CI 95%
Control	1360	37.6	35.6 – 39.6
Abdominal Obesity	1280	35.3	33.4 – 37.3
Hypertriglyceridemia	180	5.0	4.3 – 5.7
HTGW Phenotype	800	22.1	20.6 – 23.7

CI = confiance interval, HTGW = hypertriglyceridemic waist.

Table 3. Influence of abdominal obesity, hypertriglyceridemia and hypertriglyceridemic waist (HTGW) phenotype on the prevalence of hyperuricemia.

Disturbances	WC ≥ 90 cm and TG ≥ 2.0 mmol/L			WC ≥ 91 cm and TG ≥ 1.73 mmol/L		
	OR	CI 95%	*p*	OR	CI 95%	*p*
Abdominal Obesity[a]	3.7	2.5 – 5.5	< 0.0001	4.9	3.3 – 7.3	<0.0001
Hypertriglyceridemia[b]	2.8	2.2 – 3.7	<0.0001	5.8	4.3 – 7.7	<0.0001
HTGW Phenotype[c]	4.3	3.3 – 5.6	<0.0001	7.3	5.5 – 9.7	<0.0001

WC = waist circumference, TG = triglycerides and HTGW = hypertriglyceridemic waist.

[a] Model adjusted for age, HOMA-IR, sistolic blood pressure, diastolic blood pressure, ethylism, tabagism, sedentarism and serum triglycerides;

[b] Model adjusted for age, HOMA-IR, sistolic blood pressure, diastolic blood pressure, ethylism, tabagism, sedentarism and abdominal circumference;

[c] Model adjusted for age, HOMA-IR, sistolic blood pressure, diastolic blood pressure, ethylism, tabagism and sedentarism.

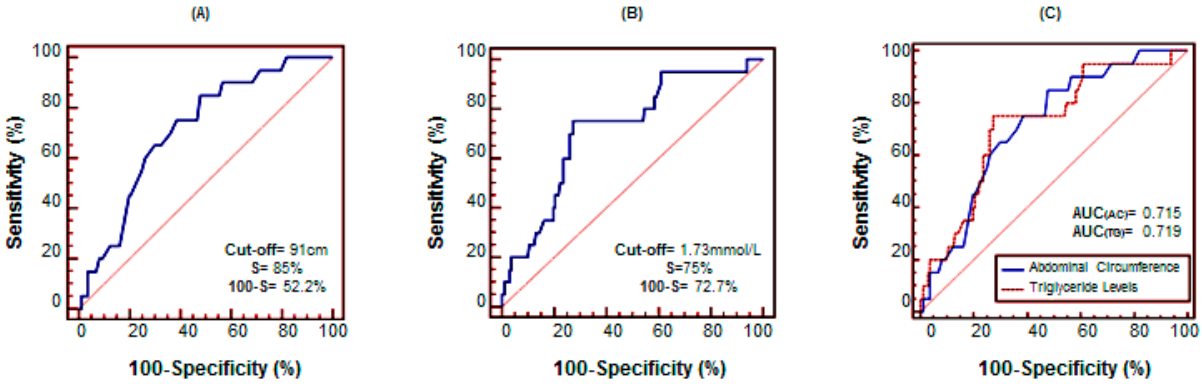


Figure 1. Receiver operating characteristics curves for the analysis to stablish the best waist circumference and triglycerides cut-off points considering the incidence of hyperuricemia. (A) waist circumference measurements; (B) triglycerides levels; (C) comparation of curves.

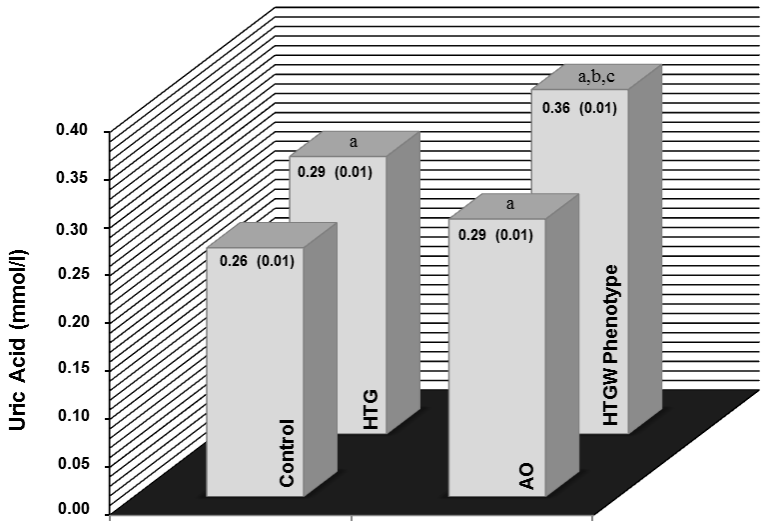


Figure 2. Serum Uric acid levels according to presence of abdominal obesity, hypertriglyceridemia and hypertriglyceridemic waist phenotype. AO = abdominal obesity, HTG = hypertriglyceridemia and HTGW = hypertriglyceridemic waist.

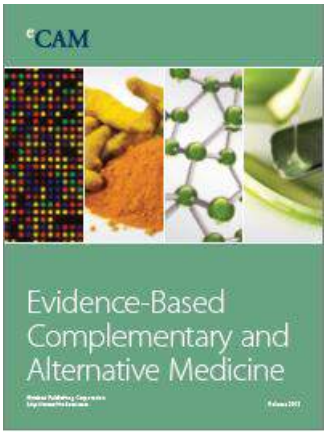
[a] Diferença significativa (p<0.0001) quando comparado ao grupo de indivíduos controle;

[b] Diferença significativa (p<0.0001) quando comparado ao grupo de indivíduos com obesidade abdominal;

[c] Diferença significativa (p<0.0001) quando comparado ao grupo de indivíduos com hipertrigliceridemia.

VI. ARTIGO 2

"Antidiabetic and Anti-hyperuricemic Effect of Acetonic Extract of *Myrciaria cauliflora* Fruit Peel"



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Antidiabetic and Anti-hyperuricemic Effect of Acetonic Extract of *Myrciaria cauliflora* Fruit Peel

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Abstract

Diabetes is a major global metabolic disorder of current century and hyperuricemia has been shown to be associated with impaired glucose metabolism. Fruits represent a vast source of potentially useful dietary supplementations for improving hyperglycemia and hyperuricemia and preventing long-term complications, but most of fruit peels are not used although they are rich in secondary products of pharmacological interest. Thus, the aim of the present study was to investigate whether acetone extract of *Myrciaria cauliflora* fruit peel is able to ameliorate diabetes and hyperuricemia. The acetone extract of *M. cauliflora* fruit peel (MCAE) was obtained, and orally administered to alloxan-induced diabetic mice at doses of 200 mg/kg/day and 400 mg/kg/day during 14 days. The MCAE was orally administered to potassium oxonate-hyperuricemic mice at both doses. The results demonstrated that fasting blood glucose levels were significantly reduced by 39% and 58% after the treatment with 200 mg/kg/day and 400 mg/kg/day, respectively. Similarly, serum levels of triglycerides, urea, creatinine, aspartate aminotransferase and alanine aminotransferase were also significantly reduced by the treatment with both doses of MCAE; and, serum levels of HDL-cholesterol were significantly increased. Furthermore, histological analysis of pancreas revealed an induction of pancreatic β cell proliferation by MCAE treatment. The results demonstrated that uric acid levels were significantly reduced by 39.7% and 49.1% after the treatment with 200 mg/kg/day and 400 mg/kg/day, respectively Therefore, this study demonstrated for the first time that *M. cauliflora* fruit peel (acetonic extract) may be a promising therapeutic source for the treatment of diabetes with natural products, and could ameliorate liver and kidney alterations caused by diabetes mellitus in mice.

Key Words: antidiabetic activity, renal function, hepatic function, lipid profile, anti-hyperuricemic activity.

1. Introduction

Diabetes mellitus is a major threat to global public health as the world wide incidences rising day by day, now emerging as global epidemic disease affecting approximately 285 million people worldwide, and it may increase to 439 million by 2030 [1]. Type 1 diabetes is an autoimmune disease caused by destruction of insulin producing beta cells when auto aggressive T-lymphocytes infiltrate the pancreas. This leads to hypoinsulinaemia and thus hyperglycemia [2]. An immediate need for exogenous insulin replacement is also a hallmark of type 1 diabetes, for which lifetime treatment is needed [3].

The discovery of insulin in 1921-22 was clearly the most significant therapeutic event in the history of type 1 diabetes; however, exogenous insulin replacement does not always provide the metabolic regulation necessary to avoid diabetes associated-complications, e.g. cardiovascular disease and hyperuricemia [3]. Some observational studies have identified that hyperuricemia could be related to the diabetes. In the context, phytotherapy is becoming more mainstream as up-to-date analysis and researches show their value in the treatment of disease [4].

Alternative approaches for the treatment of type 1 diabetes are needed and various fruits have been used in the treatment of this disease in experimental models [5-8]. The presence of antioxidants substances in fruits have been proposed as a potential candidate for searching new antidiabetic and antihyperuricemic compounds [9]. In this context, high amounts of antioxidant compounds are found in tropical fruits and phenolic compounds have demonstrated to possess this effect in various model systems [4, 10-12].

Epidemiological evidences have suggested that diets rich in edible dark-colored fruits can reduce the incidence of cardiovascular diseases and diabetes [13]. The interest in edible tropical fruits has been increased in developed countries due to their potential health benefits. In Brazil, a fruit named Jabuticaba is produced by *Myrciaria cauliflora* (Mart.) O. Berg. (Myrtaceae family), a native plant known as “Brazilian grape tree”. This fruit have a sweet pleasant taste with a little acidity, and they are consumed in natura or used to prepare wines and juices [14]. *M. cauliflora* fruit has 2.0-3.5 cm in diameter and the color of peel ranges from dark-purple to black [15]. Similar to other dark-colored fruits, jabuticaba is a

source of traditional nutrients, and also phytochemicals compounds of medicinal importance, such as polyphenols, being the latter extracted with organic solvents [16, 17].

Thus, the aim of the present study was to investigate whether acetone extract of *M. cauliflora* fruit peel is able to ameliorate diabetes and hyperuricemia.

2. Materials and Methods

2.1 Chemicals

Alloxan monohydrated and potassium oxonate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Insulin (Humolin N) was purchased from Lily (Brazil). Allopurinol was purchased from GlaxoSmithKline (Brazil). Aspartate amino transferase (AST), alanine amino transferase (ALT), creatinine, urea, total cholesterol, HDL-cholesterol, triglyceride and uric acid assay kits were obtained from Labtest Diagnostica (Brazil). Ether, acetone and all other chemicals were of the highest purity analytical grade available.

2.2 Plant Material and Preparations of Acetonic Extract

Fresh fruits of *M. cauliflora* were collected from the Limoeiro City, PE, Northeastern Brazil. The fruit peels were immediately separated, dried at room temperature (28 ± 2 ^{o}C) for 3 days, and milled to a fine powder in a Macsalab mill (Model 200 Lab; Eriez®, Bramley). The finely powdered dry fruit peels (100g) was mixed with ether (200 mL), subjected to a mechanical stirring, at 4 ^{0}C for 16 h, then filtered with Whatman filter paper (N^{o} 1) and the powder residue was mixed again with ether (200 mL) and the extraction process was repeated twice. The powder residue was mixed with acetone according to the above described procedure to yield acetone fraction. The solvent was removed by rotary evaporation (BUCHLER INSTRUMENTS, Fort Lee, NJ, USA). Acetone is a solvent with high polarity and is able to extract phenolic compounds [18]. Therefore, the *M. cauliflora* acetonic extract (MCAE) were used in the present study.

2.3 Preliminary Phytochemicals Studies

A simple qualitative and semiquantitative phytochemical analysis was performed by screening tests according to Harbone [19]. The phytochemical profile of MCAE were evaluated on thin layer chromatography plates in front of the mobile phase solvents containing different proportions and different polarities, and revealing, using as stationary phase, pre-activated GEK silica GF 254 plates (Merck). For identification and differentiation of the disclosed compounds the following parameters were used: staining band and luminescence in UV lamps.

2.4 Animals

Male Swiss white mice (*Mus musculus*), 60-days-old, weighing 30 ± 5g were obtained from the Animal House of the Laboratory of Immunopathology Keizo Asami (LIKA), UFPE, Brazil. The animals were separated into groups (N = 6) and housed in cages at a temperature of 22 ± 2 ^{0}C, 12 h dark-light cycle, relative humidity 55 ± 5%, with free access to standard chow (Labina, Purina Brazil Ltd., Brazil) and water *ad libitum*. All experimental protocols were performed according to the Animal Care and Use Committee of UFPE, Brazil, and all the experimental procedures were conducted in accordance with the ethical guidelines for the Care and Use of Laboratory Animals.

2.5 Acute Toxicity Study

Acute oral toxicity study was performed as per Organization of Economic Cooperation and Development Guidelines [20]. Health Swiss white mice were randomly divided into two groups with males and females (6 animals for group). The animals were kept fasting overnight, but with free access to water for 4 h, before oral administration of a maximum dose (1000 mg/kg) of MCAE. The animals were observed continuously for 1 h, then frequently for 4h, and later at the end of 24 h for analyzing the general behavioral, neurological and autonomic profile.

2.6 Induction of Experimental Diabetes

The animals were overnight fasted and experimental diabetes was induced by a single intraperitoneal injection of a freshly prepared solution of alloxan monohydrate (80

mg/kg) in 0.9% NaCl, then the animals were allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia [21]. After alloxan administration, all animals were relocated to their cages and given free access to food and water. Diabetes was confirmed by measuring the fasting blood glucose levels 72 h after alloxan injection. The mice with serum glucose >250 mg/dL were considered diabetic [22].

2.7 Experimental Design

The mice were split into five groups (n = 6, for group) as follows:

Group I: Normoglycemic mice receiving the vehicle saline solution (0.9%) for 14 days, as normal control group (control).
Group II: Aloxan-induced diabetic mice receiving saline solution (0.9%, orally), named diabetic nontreated (NT).
Group III: Aloxan-induced diabetic mice treated with MCAE (200 mg/kg/day, orally) in saline solution (0.9%) for 14 days, named diabetic treated 200 (MCAE [200]).
Group IV: Aloxan-induced diabetic mice treated with MCAE (400 mg/kg/day, orally) in saline solution (0.9%) for 14 days, named diabetic treated 400 (MCAE [400]).
Group V: Aloxan-induced diabetic mice treated with insulin (10 mg/kg/day, intraperitoneally) for 14 days, named diabetic insulin treated (IT).

Before and at the end of the experimental period, overnight fasting mice were anaesthetized with 2% xylazine hydrochloride (10 mg/kg) and 10% ketamine hydrochloride (115 mg/kg). Blood samples were withdrawn with a capillary from mice cavernous sinus for biochemical parameters determination, as reported previously [23]. The mice were sacrificed by cervical dislocation. Thereafter, pancreas was excised and immediately fixed in 10% neutral buffered formalin for histological analysis.

2.8 Biochemical Analysis

Mice blood samples were centrifuged at 2,500 x *g* for 15 min at 4 ^{0}C (Sorvall RC6, NC, US). Fasting blood glucose, AST, ALT, urea, creatinine, total cholesterol, HDL-

cholesterol and triglycerides were measured by enzymatic colorimetric assays using commercial kits according to the manufacturer's instructions.

2.9 Histological Analysis of Pancreas

Pancreas from all groups was subjected to standard routine tissue processing technique: dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections of 5μm thickness were cut from each block and stained with haematoxylin-eosin for histological examination. Prepared slides were studied by light microscopy and all sections were evaluated for the tissue injury.

2.10 Induction of Hyperuricemia

Hyperuricemic mice model induced by potassium oxonate (uricase inhibitor) was used (Hu et al., 2010). Mice were orally injected with potassium oxonate (250 mg/kg) 1 h before the final extracts or drug administration to increase serum uric acid levels. Food, but not water, was withdrawn from the animals 1h prior to extract or drug administration.

The mice were split into five groups (n = 6, for group) as follows:

Group I: Normouricemic mice receiving the vehicle saline solution (0.9%) for 7 days, as non hyperuricemic group (NHU).
Group II: Potassium oxonate-induced hyperuricemic mice receiving saline solution (0.9%, orally), named hyperuricemic nontreated (HU).
Group III: Potassium oxonate-induced hyperuricemic mice treated with MCAE (200 mg/kg/day, orally) in saline solution (0.9%) for 7 days, named hyperuricemic treated 200 (MCAE [200]).
Group IV: Potassium oxonate-induced hyperuricemic mice treated with MCAE (400 mg/kg/day, orally) in saline solution (0.9%) for 7 days, named hyperuricemic treated 400 (MCAE [400]).

Group V: Potassium oxonate-induced hyperuricemic mice treated with allopurinol (10 mg/kg/day, orally) in saline solution (0.9%) for 7 days, named hyperuricemic allopurinol treated (ALP).

In the final of experiment blood samples were collected from mice with a capillary from mice cavernous sinus. The blood was centrifuged at 2,500 x *g* for 15 min at 4 ^{0}C to obtain the serum. The levels of uric acid were measured using standard diagnostic kits according to the manufacturer's instructions.

2.11 Statistical Analysis

Results were presented as the mean ± SD. The statistical analysis involving multiple comparisons were analyzed by one-way ANOVA followed by Tukey's *post hoc* test using software PRISMA (GraphPad Software, Inc., San Diego, CA, version 5.01). Values of $P < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Phytochemical Constituents

As demonstrated by the phytochemical analysis, the highest phenolic compounds in MCAE are flavonoids, phenylpropanoids, followed by proanthocyanidins. Several studies have highlighted the presence of different phenolic compounds in fruit peels and its antioxidants potential [5-8]. The efficacy of antioxidants as a tool to treat some low to mild and chronic disorders with very low rates of progression has been widely documented in the literature [8]. Various pharmacological studies have emphasized the potential of fruits intake, in regular diet, in health promoting, due to the presence the phenolic compounds that also act as natural antioxidants [16, 24, 25]. Hence, natural antioxidants have been shown to possess antidiabetic potential against different experimental systems, such as diabetes induced by streptozotocin or alloxan [26]. Thus, the presence of phenolic compounds in MCAE indicate that this extract may a good source of flavonoids, phenylpropanoids, and proanthocyanidins for potential application.

3.2 Antidiabetic Effect of MCAE

Diabetes is a chronic disease and alterations in carbohydrate metabolism and several organ injuries are found in the body with this pathology [22]. The type 1 of diabetes is characterized by destruction of pancreas and modulates the decreasing of insulin release and promotes hyperglycemia. Individuals with this disease become dependent on exogenous insulin, which difficult the treatment and control of alterations introduced by disease [3]. Type 1 diabetes has been developed in experimental models by the administration of alloxan. This drug promotes excellent experimental diabetes to generate reactive species that induce selective destruction of beta cell of pancreas by necrosis [21]. Thus the administration of a single dose of alloxan in animals in this study effectively promoted metabolic changes of diabetes.

In diabetic groups of this study, the levels of fasting blood glucose were significantly (P<0.0001) higher than that of normal control group. The administration of MCAE in a dose of 200 mg/kg/day and 400 mg/kg/day for 14 days significantly lowered the blood glucose levels in a dose dependent manner (Figure 1). Administration of a dose of 400 mg/kg/day exhibited better glucose reduction (58%) than 200 mg/kg/day (39%). The reduction on blood glucose observed for the mice treated with the highest dose of MCAE tested was lower to that found with insulin treatment (73%). Our results are in agreement with several studies that reports the improvement in hyperglicemia by treatment with fruit extracts [12, 27, 28].

Previous studies showed that phenolic compounds present in extracts of fruits acted on ATP sensitive K^+ channels [29] and regulate blood glucose. The hypoglycemic effect of fruit extracts may be due to the potentiation by the pancreatic secretion of insulin from regenerated β cells [28]. We can suppose that the presence of phenolic compounds in MCAE may be responsible for the hypoglycemic activity observed in alloxan-induced diabetic mice. In accordance with our results, studies with fruit extracts of *Artocarpus heterophylus* [12], *Mormodica charantia* [30], *Solanum torvum* [28], *Psidium guajava* [31], *Curculigo latifolia* [32] and *Tamarindus indica* [10] demonstrated the potential antidiabetic of the fruits.

Therefore, the antidiabetic effect of MCAE suggests that the jabuticaba has a therapeutic potential for decreasing blood glucose and treating experimental diabetes.

3.3 Effect of MCAE on Lipid Profile, and Liver and Kidney Function

As shown in figure 2, total cholesterol levels in animals treated with MCAE (200 mg/kg and 400 mg/kg) and with insulin were not significantly different from that found in the control group, unlike the diabetic nontreated animals that significantly increased total cholesterol concentration. In turn, HDL-cholesterol levels were significantly increased by the treatment with MCAE 200 mg/kg (12.7%) and 400 mg/kg (24.5%), and also, the treatment with insulin increased HDL-cholesterol levels (29%), as demonstrated in Figure 3. The increase of HDL-cholesterol in diabetic mice reported in this study are in agreement with recent report by Aswar & Kuchecar [27] and Ishak et al. [32], who demonstrated that HDL-cholesterol levels improve in diabetic mice after treatment with extracts of fruits.

It is well known that HDL-cholesterol levels are an important cardiovascular protector factor [22]. HDL-cholesterol is known to be lowered during diabetes and have been implicated in the development of atherosclerosis [33]. Dietary polyphenols have been shown to pocess cardioprotective effects by altering hepatic cholesterol absorption, the processing of lipoproteins in plasma, and inflammation [34]. Baba et al., [35] reported that continuous intake of phenolic compounds exerts a beneficial effect on plasma HDL-cholesterol concentration. Thus, phenolic compounds presents in MCAE may contribute to an elevation in HDL-cholesterol and this indicates an improvement of the lipid metabolism associated to a cardioprotective effect in diabetic mice.

Figure 4 shows that the levels of triglycerides were significantly increased in alloxan-induced diabetic mice, in comparison with normal mice. However, after 14 days of treatment with MCAE at doses of 200 mg/kg and 400 mg/kg, the triglyceride concentrations in sera of diabetic mice decreased, significantly ($P < 0.0001$) by 40.3% and 49.9%, respectively, and this result was similar to the treatment with insulin that reduced triglyceride levels by 51.1%. Our results are in agreement with reports by Hossaine et al. [30] and Koyagura et al. [10], who demonstrated that hypertriglyceridemia can be ameliorated by treatment with extracts of medicinal plants.

Alterations in lipid profile are one of the most common complications in diabetes and found in 40% of diabetic cases [33]. As demonstrated in this work, total cholesterol

levels of diabetic mice treated with MCAE did not differ significantly from that of the control group, unlike the diabetic nontreated mice. Moreover, MCAE treatment presented a relevant hypotriglyceridemic effect in diabetic mice treated with both doses 200 mg/kg and 400 mg/kg. Hypertrilglyceridemia in diabetes can result from an impaired catabolism of triglycerides rich lipoproteins. This can be explained because the function of lipoprotein lipase, the key enzyme in removal and degradation of triglycerides in attenuated by insulin deprivation [37]. Phenolic compound-rich food have been suggested to be involved in the treatment of hypertriglyceridemia [38, 39]. Yoshikawa et al. [40] reported that orally administered phenolic compounds extracted from *Salacia reticulata* lowered lipoprotein lipase and serum triglycerides. Because MCAE contains phenolic compounds including flavonoids, phenylpropanoids and proanthocyanidins, we suggest that the presence of compounds presents in this extract have been exerting hypotriglyceridemic effect in experimental diabetes.

As shown in the Table 2, levels of AST and ALT, enzymes known as biochemical markers of hepatic function, and urea and creatinine, two biochemical markers of renal function, were significantly increased in alloxan-induced diabetic mice, in comparison with control. AST and ALT were significantly reduced after treatment with 200 mg/kg and 400 mg/kg; the activity of AST was reduced by 32.7% and 48.3%, respectively, and ALT activity by 28.7% and 49.3%, with 200 mg/kg and 400 mg/kg. After the treatment with MCAE at doses of 200 mg/kg and 400 mg/kg, serum levels of urea and creatinine also were reduced. Urea was reduced by 23.3% and 35% respectively; and creatinine concentrations by 10% and 12.5%, respectively. The reduction of AST, ALT, urea and creatinine were similar to those observed with insulin treatment.

Previous studies have reported that hepatic damage in alloxan-induced diabetic mice can be improved by decreasing the levels of serum AST and ALT after herbal bioactive agents [22]. Liver diseases are an important problem of health and the release of intracellular enzymes such as AST and ALT into the blood indicates hepatocytes damage [41, 42]. Furthermore, elevations in AST and ALT in NT group indicate that diabetes induced by alloxan promotes live damage and loss of integrity of the hepatocyte membranes. In addition, restorative capacities were attributed to the phenolic compounds when normal levels of serum AST and ALT were achieved in impaired hepatic function after treatment with polyphenolic extracts [43]. Thus, the reduction in serum levels of AST

and ALT in diabetic mice after treatment with MCAE (200 and 400 mg/kg) indicates the effectiveness of this extract in treating liver damage promote by diabetes.

Diabetes is usually related with reduction of renal function. The kidney damage in diabetes is associated with the presence of hypertrophy of the glomeruli and tubular cells, and enhanced renal blood flow [44, 45]. Serum elevations in urea and creatinine levels are usually reported as effectiveness biochemical markers of kidney damage [46]. There are strong experimental evidences that show that diabetes promotes renal damage by the ability to increase the free radicals formation in the kidneys [47, 48]. In the context, polyphenolic-rich extract of *Vaccinium myrtillus* reduced the degree of stress oxidative and kidney damage [49]. Thus, the presence of phenolic compounds in MCAE may contribute a reduction in urea and creatinine levels and our results clearly indicate that MCAE possesses a potential to ameliorate renal function in diabetic mice.

3.4 Effects of MCAE on the Histopathological Changes of the Pancreas

The structure of the pancreas of the control and diabetic mice are shown in Figure 4. Pancreas of control group showed normal pancreatic islet of Langerhans and acinar cells (Figure 4(a)). By contrast, in alloxan-induced diabetic mice the acinar cells were altered with presence of vacuoles; furthermore deterioration of pancreatic islets was also observed (Figure 4(b)). MCAE (200 mg/kg/day) treatment increased the number of pancreatic islets as compared to that of diabetic nontreated animals (Figure 4(c)). Interestingly, pancreatic section of diabetic mice treated with MCAE (400 mg/kg/day) showed pancreatic islet similar to that of the control group (Figure 4(d)).

The histopathological analysis of pancreas isolated from mice administrated with alloxan alone revealed tissue damage with deterioration of pancreatic islets. In this connection, it may be observed that several authors reported such changes in pancreas tissues of mice exposed to prominent diabetogenic alloxan for its ability to induce reactive oxygen species (ROS) formation, resulting in the selective necrosis of beta cells in pancreatic islets [21]. However, the diabetic animals treated with MCAE showed normal architecture of pancreatic tissue, suggesting the regeneration of pancreatic islet by MCAE administrations. The ability of acetonic extracts of fruits of *Momordica charantia* to stimulate pancreatic growth has been reported [50]. The regenerative action of MCAE

corroborates with acetonic extract of *Momordica charantia.* Thus, the antidiabetic effect observed by MCAE administration suggests the therapeutic potential in preventing and/or treating histological alteration in diabetes.

3.5 Anti-hyperuricemic Effect of MCAE

Figure 5 summarized the anti-hyperuricemic effect of MCAE in potassium oxonate-induced hyperuricemic mice. Levels of serum uric acid in hyperuricemic mice treated with potassium oxonate were significantly (P<0.0001) increased compared with control mice. MCAE at doses 200 and 400 mg/kg significantly lowered levels of uric acid in hyperuricemic mice in 39.7% and 49.1%, respectively. Allopurinol at 10 mg/kg also markedly reduced the levels of uric acid (45.9%).

Hyperuricemia is a risk factor resulting in gout and other chronic diseases. But, currently the therapeutic agents for lowering serum uric acid are sometimes limited due to the associated undesirable adverse effects. A potential source of new anti-hyperuricemic agents may be derived from the natural products [51]. In this context, MCAE may be a natural product with potential anti-hyperuricemic. Previous studies have demonstrated the potential anti-hyperuricemic of phenolic compounds [52]. While it has been shown that some flavonoids can change xanthine oxidase activities and serum uric acid levels in vitro and in vivo [53-55]. Xanthine oxidase catalyses the oxidation of hypoxanthine and xanthine to uric acid and therapeutic agents for inhibition of enzyme as they could be used to block the biosynthesis of uric acid [56]. Huang et al., [52] demonstrated the potential of flavonoids in inhibition of xanthine oxidase activities. We can suppose that the presence of flavonoids in MCAE may be responsible for the anti-hyperuricemic activity observed in potassium oxonate-induced hyperuricemic mice. Thus, the anti-hyperuricemic effect observed by MCAE administration suggests the therapeutic potential in treating hyperuricemia.

3.6 Acute Toxicity of MCAE

Oral administration of the MCAE did not produce significant changes in behaviors. There were no changes in the nature of stool, urine and eye color. No mortality was observed post administration of MCAE to the end of the experiment. In a single dose

administration no adverse effects was observed for the acetonic extract of *M. cauliflora*, indicating that the extracts are not toxic under the observable condition. Thus, MCAE present active compounds which may be used for diabetes treatment without evidence of toxicity.

4. Conclusion

Our results indicate that oral administration of MCAE ameliorates the hyperglycemia in diabetic mice, not only reducing values of blood glucose, but most of all MCAE ameliorates the metabolic injuries that diabetic mice suffered in consequence of hyperglycemia, the dyslipidemia, and the liver and kidney disorders. It is worth noting that MCAE is a good source of natural antioxidants, which could be a valuable tool in controlling the biochemical and histological alterations in alloxan-induced diabetic mice.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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Table 1. Phytochemical screening of MCAE.

Tests	Result
Alkaloids (*Dragendorf*)	-
Steroids (*Lieberman Buchard*)	-
Coumarins (*UV*)	-
Flavonoids (*Neu*)	+++
Proanthocyanidins (*Vanilin Hydrochloric*)	++
Phenylpropanoids (*UV*)	+++
Irydoids (*Vanilin Sulfuric*)	-
Triterpens (*Lieberman Buchard*)	-

(-) non detected; (+) Present in low concentration; (++) present in moderate concentration; (+++) Present in high concentration.

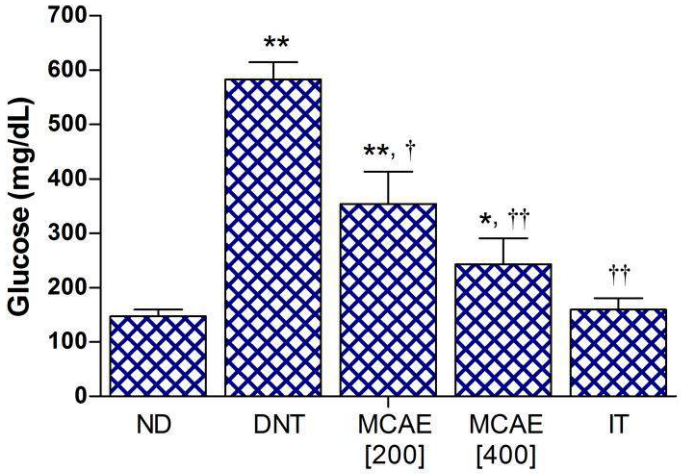

Figure 1. Effect of MCAE on fasting blood glucose levels of the control and treated animals. ND – non diabetic; DNT – diabetic nontreated alloxan-diabetic mice; MCAE [200] – alloxan-diabetic mice treated with MCAE (200 mg/kg/day); MCAE [400] – alloxan-diabetic mice treated with MCAE (400 mg/kg/day); IT – alloxan-diabetic mice treated with insulin (10 mg/kg/day). The values represent the mean ± SD. *$P < 0.05$ versus control; **$P < 0.0001$ versus control, $^{\dagger}P < 0.05$ versus NT; $^{\dagger\dagger}P < 0.0001$ versus NT.

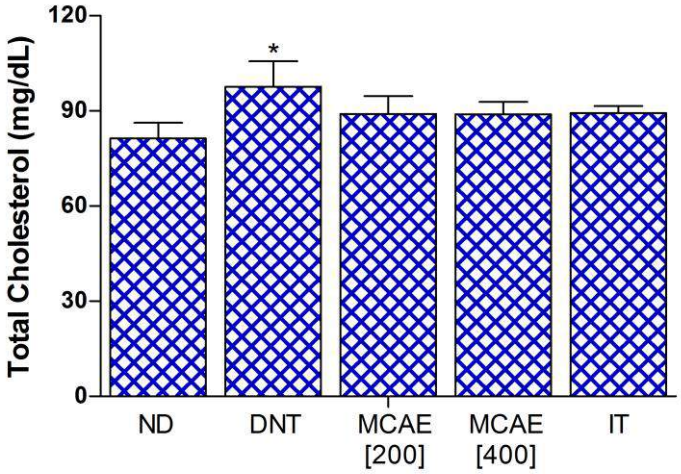

Figure 2. Effect of MCAE on total cholesterol of the control and treated animals. ND – non diabetic; DNT – diabetic nontreated alloxan-diabetic mice; MCAE [200] – alloxan-diabetic mice treated with MCAE (200 mg/kg/day); MCAE [400] – alloxan-diabetic mice treated with MCAE (400 mg/kg/day); IT – alloxan-diabetic mice treated with insulin (10 mg/kg/day). The values represent the mean ± SD. One-way ANOVA with Tukey's *post hoc* test. *$P < 0.05$ versus control.

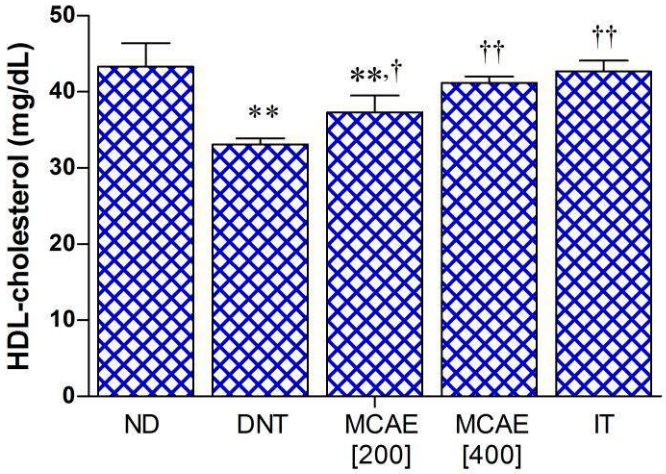

Figure 3. Effect of MCAE on HDL-cholesterol of the control and treated animals. ND – non diabetic; DNT – diabetic nontreated alloxan-diabetic mice; MCAE [200] – alloxan-diabetic mice treated with MCAE (200 mg/kg/day); MCAE [400] – alloxan-diabetic mice treated with MCAE (400 mg/kg/day); IT – alloxan-diabetic mice treated with insulin (10 mg/kg/day). The values represent the mean ± SD. One-way ANOVA with Tukey's *post hoc* test. *$P < 0.05$ versus control; **$P < 0.0001$ versus control, $^{\dagger}P < 0.05$ versus NT; $^{\dagger\dagger}P <$ 0.0001 versus NT.

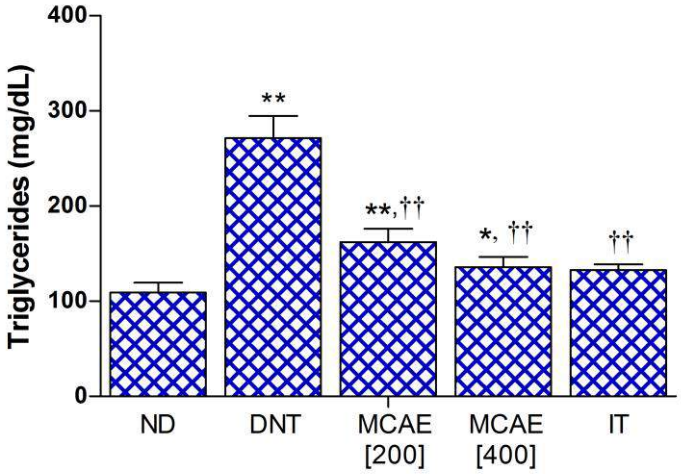

Figure 4. Effect of MCAE on triglycerides of the control and treated animals. ND – non diabetic; DNT – diabetic nontreated alloxan-diabetic mice; MCAE [200] – alloxan-diabetic mice treated with MCAE (200 mg/kg/day); MCAE [400] – alloxan-diabetic mice treated with MCAE (400 mg/kg/day); IT – alloxan-diabetic mice treated with insulin (10 mg/kg/day). The values represent the mean ± SD. One-way ANOVA with Tukey's *post hoc* test. *$P < 0.05$ versus control; **$P < 0.0001$ versus control, $^{\dagger\dagger}P < 0.0001$ versus NT.

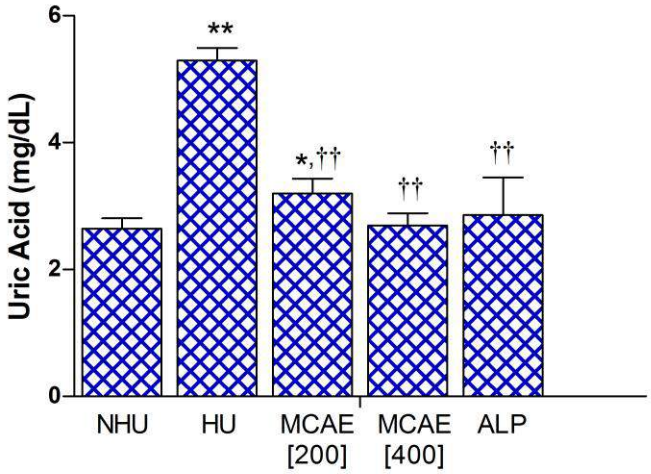

Figure 5. Effect of MCAE on uric acid levels of the control and treated animals. NHU – non hyperuricemic mice; HU – potassium oxonate-hyperuricemic mice; MCAE [200] – potassium oxonate-hyperuricemic mice treated with MCAE (200 mg/kg/day); MCAE [400] – potassium oxonate-hyperuricemic mice treated with MCAE (400 mg/kg/day); ALP – potassium oxonate-hyperuricemic mice treated with allopurinol (10 mg/kg/day). The values represent the mean ± SD. One-way ANOVA with Tukey's *post hoc* test. *$P < 0.05$ versus control; **$P < 0.0001$ versus control, $^{\dagger\dagger}P < 0.0001$ versus NT.

Table 2. Hepatic and renal parameters of the control and treated animals.

GROUPS	AST (U/L)	ALT (U/L)	Urea (mg/dL)	Creatinine (mg/dL)
ND	103.3 ± 4.6	42.0 ± 3.5	32.9 ± 2.5	0.32 ± 0.01
DNT	207.3 ± 15.0**	87.2 ± 12.9**	55.4 ± 7.8**	0.40 ± 0.02**
MCAE [200]	139.5 ± 8.2*,††	62.2 ± 7.6**,††	42.5 ± 5.8**	0.36 ± 0.02**,†
MCAE [400]	107.2 ± 9.6††	44.2 ± 3.7††	36.0 ± 4.5††	0.35 ± 0.01††
IT	108.8 ± 9.4††	44.2 ± 3.7††	39.2 ± 2.9††	0.34 ± 0.01††

AST – Aspartate Aminotransferase; ALT – Alanine Aminotransferase; ND – non diabetic; DNT – diabetic nontreated alloxan-diabetic mice; MCAE [200] – alloxan-diabetic mice treated with MCAE (200 mg/kg/day); MCAE [400] – alloxan-diabetic mice treated with MCAE (400 mg/kg/day); IT – alloxan-diabetic rats treated with insulin (10 mg/kg/day). The values represent the mean ± SD. One-way ANOVA with Tukey's *post hoc* test. $^{*}P < 0.05$ versus control; $^{**}P < 0.0001$ versus control, $^{\dagger}P < 0.05$ versus NT; $^{\dagger\dagger}P < 0.0001$ versus NT.

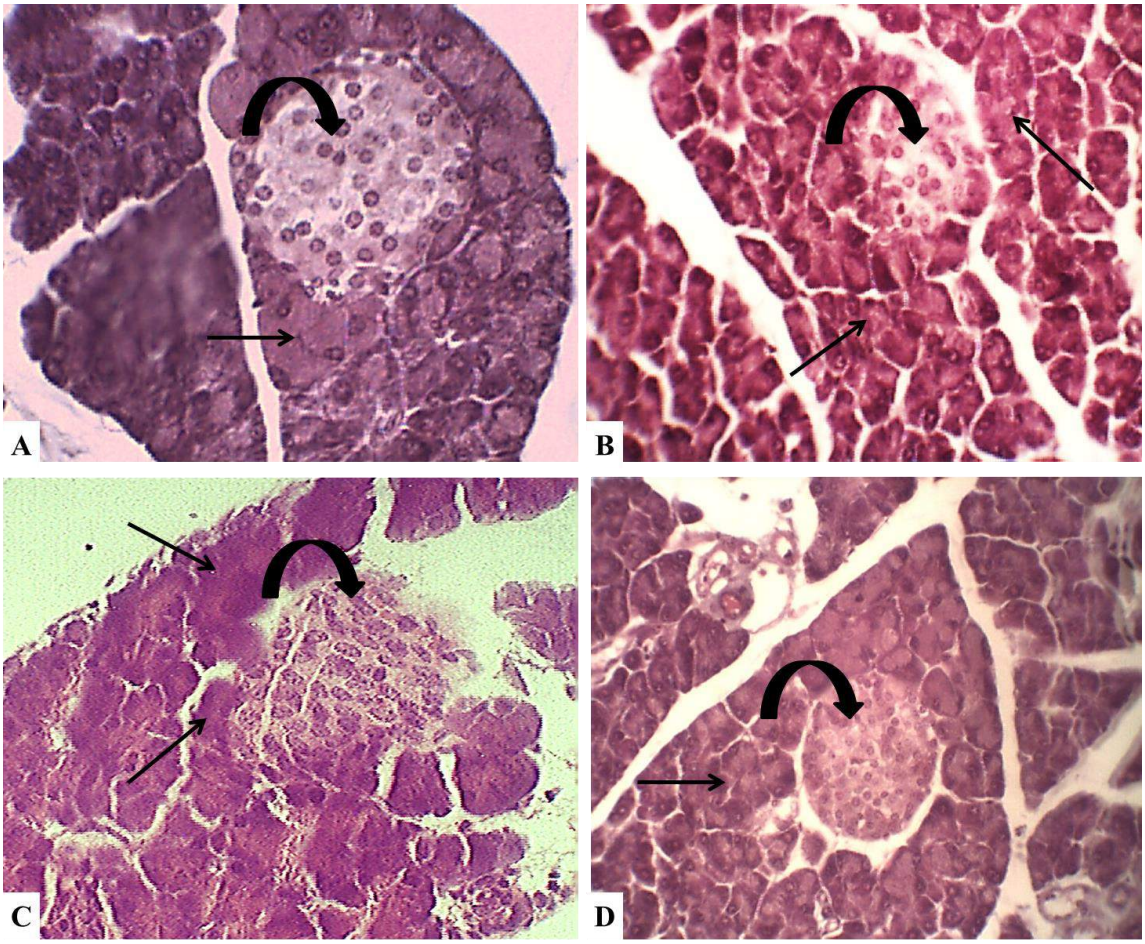

Figure 6. Histopathological changes in pancreatic tissue. (A) non diabetic group – preserved pancreatic islet of Langerhans (curved arrow) and preserved pancreatic acinar cells (straight arrows). (B) diabetic non treated – atrophic pancreatic islet of Langerhans with few cells (curved arrow) and the presence of some vacuoles in pancreatic acinar cells (straight arrows). (C) diabetic treated with MCAE (200 mg/kg/day) – pancreatic islet of Langerhans (curved arrow) and pancreatic acinar cells in disorder. (D) diabetic treated with (400 mg/kg/day) – preserved pancreatic islet of Langerhans (curved arrows) and preserved pancreatic acinar cells (straight arrows). Haematoxylin-Eosin: 400x.

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VII. ARTIGO 3

"Biological Activities of Organic Extracts of *Myrciaria cauliflora* Fruit Peel"

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Biological Properties of Organic Extracts of *Myrciaria cauliflora* Fruit Peel

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ABSTRACT

Jaboticaba is a dark-colored fruit produced by *Myrciaria cauliflora* (Mart.) O. Berg., a native plant known as "Brazilian grape tree". The objective of the present study was to evaluate the anti-inflammatory, antinociceptive and antioxidant properties of organic extract of fruit peel of *M. cauliflora*. Organic extracts of *M. cauliflora* were prepared using standard methods and the ether extract (MCEE), acetone extract (MCAE) and methanol extract (MCME) were obtained. Anti-inflammatory activity was evaluated through oedema paw ad peritonitis test in male mice. Antinociceptive activity was assessed in male mice using abdominal writhing and hot plate test. Antitumoral activities were evaluated against solid tumor of Ehrlich carcinoma. The antioxidant activity of all extracts was evaluated using a 2,2—diphenyl-β-picrylhydrazyl (DPPH) assay. All organic extracts presented too antioxidant activities. The orally treatment with 200 and 400mg/kg of MCAE and MCME produced significant inhibition on inflammation for peritonitis (MCAE: 35% and 45%, respectively; MCME: 45% and 54%, respectively). The MCAE and MCME extracts (200 and 400mg/kg, orally) produced significant inhibition on inflammation induced by carrageenan for oedema paw at both doses (MCAE: 93.5% and 83.7%, respectively; MCME: 96.7% and 95.9%, respectively). All organic extracts produced significant inhibition on antinociceptive from abdominal writhing test and hot plate tests with orally treatment of 200 and 400mg/kg. All organic extracts produced significant reduction on tumor weight. Taking into account the above results, it can be concluded that *M. cauliflora* has significant anti-inflammatory, antinociceptive, antitumoral and antioxidant properties, and this study provide pharmacological evidence for a potential target in pain and inflammation treatment.

Keywords: *Myrciaria cauliflora,* fruit peel, inflammation, nociception and tumor.

HIGHLIGHTS

1. Models of inflammation, and nociception solid tumor were induced in mice.
2. Extract fractions of *M. cauliflora* fruit peel were used for treatment of mice.
3. Phytochemical and antioxidant activity of extracts were determined.
4. Antioxidant compounds were identified in organic extracts of *M. cauliflora*.
5. Organic extracts exerts an anti-inflammatory, antinociceptive and antitumoral effects.

1. INTRODUCTION

Inflammatory reaction is one of the most important host defense mechanisms to eliminate or limit the spread of injurious agent (Hossein et al., 2015; Andekar et al., 2012). However persistent inflammation leads to tissue damage, pain and possibly failure of organs (Liao et al., 2012). Proinflammatory cytokines are produced in large quantities by activated macrophages that stimulate cellular responses via increasing prostaglandins (Loram et al., 2007). Free radicals and related reactive species are strongly associated with several physiopathological process including cell death, inflammation and pain (Campêlo et al., 2011). Thought, the complex events and mediators involved in the inflammatory reaction can induce or aggravate many reaction with oxidative damage and pain (Sosa et al., 2002).

Oxidative stress is also associated with cancer progression. Cancer is a group of different diseases, characterized by uncontrolled cellular growth, tissue invasion and metastases (Dashora et al., 2011). This is one leading causes of death in world and accounted for 7.9 million deaths in 2007, with 38% in developed countries and 62% in developing countries. Projections by 2030 show that almost 21.4 million new cancer cases and more 13.2 million deaths will occur worldwide (GLOBOCAN, 2008). In Brazil, a developing country, cancer is responsible for 172,044 deaths in 2010 (INCA, 2010).

Historically, human have used medicinal plants as a traditional way of providing improve to several pathological states. In this context, a crescent number of medicinal plants have been investigated for their possible anti-inflammatory, antioxidant, antitumoral and antinociceptive properties (Hossen et al., 2015; Medda et al., 2015; Sengar et al., 2015; Adzu et al., 2014 Carro et al., 2015). The use of this effective strategy for the promotion of human health has significantly increased as notable progress has been made concerning the development of natural therapies (Campelo et al., 2011). Interestingly, the World Health Organization (WHO) has recommended the integration of traditional medicines proved to be useful into national health care programs, and developed a strategy to address issues of policy, safety, efficacy, quality, access and rational use (Adzu et al., 2014).

The interest in edible tropical fruits has been increased in developed countries due to their potential health benefits (Medda et al., 2015). In Brazil, a fruit named jaboticaba is produced by *Myrciaria cauliflora* (Mart.) O. Berg. (Myrtaceae family), a native plant known as "Brazilian grape tree" (Duarte et al., 2012). This fruit have a sweet pleasant taste with a little acidity, and they are consumed in natura or used to prepare wines and juices (Wu et al., 2013a). *M. cauliflora* fruit has 2.0-3.5 cm in diameter and the color of peel ranges from dark-purple to black (Wu et al., 2013b).

The ability of some fruit-derived nutrients to reduce the risk of diseases has been related, at last in part, with the occurrence of bioactive compounds that are know to exert a wide range of biological activities (Carro et al., 2015). The secondary metabolites from plant fruits are good sources for anti-inflammatory, antinociceptive and antioxidant therapy and are a wide range of phytochemicals which acts as key tool in phytomedicine research in recent years (Medda et al., 2015). Similar to other dark-colored fruits, jaboticaba is a source of traditional nutrients, ingredients, and also secondary metabolites of medicinal importance, such as phenolic compounds, being the latter extracted with organic solvents (Chen et al., 2012; Kubola & Siriamornpun, 2011).

Several studies have highlighted the presence of different phenolic compounds in fruit peels and its antioxidants potential (Hassan et al., 2011; Contrerás-Caldeirón et al., 2011; Hetzroni et al., 2011; Ismail et al., 2012). Hence, natural antioxidants have been shown to possess anti-inflammatory, antitumoral and antinociceptive potential against different experimental models of inflammation, tumor and pain. Thus, the objective of the

present study was to evaluate the anti-inflammatory, antinociceptive and antioxidant properties of organic extract fractions of fruit peel of *M. cauliflora*.

2. MATERIAL AND METHODS

2.1 Chemicals

Acetyl Salicylic Acid, λ-carrageenan and 2,2-diphenyl-1-picrylhydrazyl was purchased from Sigma Chemicals Co. (St. Louis, MO, USA). Acetic Acid was purchased from Merck (Damstadt, Germany). Solvents used for preparation in the organic extract fractions (ether, acetone and methanol) were purchased from Vetec (Rio de Janeiro, RJ, Brazil).

2.2 Plant Material and Preparations of Organic Extracts

Fresh fruits of *M. cauliflora* were collected from the Limoeiro City, PE, Northeastern Brazil. The fruit peels were immediately separated, dried at room temperature (28 ± 2 ^{o}C) for 3 days, and milled to a fine powder in a Macsalab mill (Model 200 Lab; Eriez®, Bramley). The finely powdered dry fruit peels (100g) was mixed with ether (200 mL), subjected to a mechanical stirring, at 4 ^{0}C for 16 h, then filtered with Whatman filter paper (Nº 1) and the powder residue was mixed again with ether (200 mL) and the extraction process was repeated twice. The powder residue was mixed with acetone according to the above described procedure to yield acetone fraction. The solvent was removed by rotary evaporation (BUCHLER INSTRUMENTS, Fort Lee, NJ, USA). Therefore, the *M. cauliflora* etheric, acetonic and methanolic extracts were named MCEE, MCAE and MCME, respectivelly and all extracts were used in the present study.

2.2.2 Phytochemical Analysis

A simple qualitative and semiquantitative phytochemical analysis was performed by screening tests according to [Harbone, 1998]. The phytochemical profile of MCEE, MCAE and MCME were evaluated on thin layer chromatography plates in front of the mobile phase solvents containing different proportions and different polarities, and revealing, using as stationary phase, pre-activated GEK silica GF 254 plates (Merck). For identification and differentiation of the disclosed compounds the following parameters were used: staining band and luminescence in UV lamps.

2.3. Animals

Male Swiss white mice (*Mus musculus*), 60-days-old, weighing 30 ± 5g were obtained from the Animal House of the Laboratory of Immunopathology Keizo Asami (LIKA), UFPE, Brazil. The animals were separated into groups (N = 6) and housed in cages at a temperature of 22 ± 2 ^{0}C, 12 h dark-light cycle, relative humidity 55 ± 5%, with free access to standard chow (Labina, Purina Brazil Ltd., Brazil) and water *ad libitum*. All experimental protocols were performed according to the Animal Care and Use Committee of UFPE, Brazil, and all the experimental procedures were conducted in accordance with the ethical guidelines for the Care and Use of Laboratory Animals.

2.4 Assay of Anti-inflammatory Activity by Carrageenan-induced Paw Oedema

The paw edema was induced from a subplantar injection of 0.1 ml carrageenan (1%) in saline half hour before the administration of the extracts (Winter et al., 1962). Dose of 200 and 400 mg/kg (orally) of organic extracts of *M. cauliflora* (MCEE, MCAE and MCME) was chosen because it has best results. The volume of the paw was measured by one caliper rule (Kanon-Staineless Mardened), at the time 0 and intervals of 1, 2, 3 and 4 h immediately after the subplantar injection of carrageenan. For the positive control group, animals received a dose 100mg/kg Acetylsalicylic acid (ASA). The negative control

animals received just vehicle (saline solution 0.9%). The data obtained for the various groups were reported as mean ± S.D. and expressed in millimeter. The percentage inhibition was calculated using the formula given below, that represents the period of peak edema (3h). Percentage inhibition (%) = [(Vf-Vi) Control group mean – (Vf-Vi) Test group mean / (Vf-Vi) Control group mean] x 100, where Vf and Vi represent the volume of the initial and final paw.

2.5 Assay of Anti-inflammatory Activity by Carrageenan-induced Peritonitis

Peritonitis was conducted as described by Foster et al. (1986). Male Swiss mice (6 animals per group) were pre-treated with vehicle (Negative Control – saline solution 0.9%, orally), Acetylsalicylic acid (Positive Control - ASA, 100 mg/kg, orally), and different organic extracts of *M. cauliflora* (200 and 400 mg/kg, orally), and 1h later, the animals received an injection of 1% carrageenan (i.p.). After 4 h, the animals were sacrificed. After, saline containing EDTA (1mM, i.p.) was injected, immediately a brief massage was done for further fluid collection and used for leukocyte (mainly neutrophils) counting in a Cell Counter (ABX MICROS 60). The results were expressed as the number of Leukocytes x $10^3/mm^3$. The percentage of the leukocyte inhibition = (1 – T/C) x 100, where T represents the treated groups leukocyte counts and C represents the control group leukocyte counts.

2.6 Assay of Antinociceptive Activity by Acetic Acid-induced Abdominal Writhing

Abdominal writhing based of a contraction of the abdominal muscle together with a stretching of the hind limbs, induced by agent nociceptive (0.8% Acetic acid) with intraperitoneal injection (Koster et al., 1959). The animals received 200 and 400 mg/kg of organic extracts of *M. cauliflora* (test group), ASA (100 mg/kg, positive control group) and vehicle (negative control group) 1 hour before administration of acetic acid (0.8%, i.p). The number of writhing reflexes was counted during the following 20 min.

2.7 Assay of Antinociceptive Activity by Hot Plate

The central analgesic activity of *M. cauliflora* against thermal stimuli was studied in male mice using the hot plate test (MacDonald et al., 1946). Mice were individually placed in a hot plate heated at fixed temperature (55 ± 1.0 ° C) and response time to the stimulus was marked by a timer. Measurements were performed at time 0, 30, 60, 90, and 120 min after the first thermal stimulus. The maximum stay of the animal was 60s to avoid damage. The control group was treated with vehicle (saline solution 0.9%) and the test group (n = 6 each) with 200 and 400 mg/kg (orally) of different organic extracts of *M. cauliflora* 1 hour before performing the experiments. ASA (100mg/kg, orally) was administered to control positive.

2.8 Assay of Antioxidant Activity of Extract Fractions of M. cauliflora

The method was carried out as described by Brand, Cuvelier and Berset (1995). Various concentrations (50, 100, 200 and 500 µg/mL) of the *M. cauliflora* organic fractions were used. The DPPH solution (150 µM) was prepared with methanol solvent. The assay mixture contained in total volume of 1 mL (100 µL of the M. cauliflora organic extracts + 100µL of DPPH solution) α-tocopherol was used as the positive control. After 30 min incubation at 25 °C, the absorbance was measured at 490 nm (Biotek instruments, Inc., Winooski, VT). The radical scavenging activity was calculated:

$$\% \text{ Scavenging} = ((A_{control} - A_{sample}) / A_{control}) \times 100$$

2.9 Assay of Antitumoral Activity of Organic Fractions of M cauliflora

Ehrlich carcinoma (EC) cells were supplied by the Department of Antibiotics, UFPE, Brazil. The cells were maintained *in vivo* in mice by intraperitoneal transplantation. EC cells aspirated from the peritoneal cavity of mice were washed with saline and injected subcutaneously to develop solid tumor. For the experimental tumor induction mice were injected with EC (5.0×10^7), subcutaneously in the left footpad to obtain the solid tumor (Stock, 1955**).**

The mice (n=36) were divided into 6 groups (n=6, for group) as follows:

Group (I) – Control mice without induction of tumor, receiving saline solution (0.9%), named normal control (NC);

Group (II) – Control mice with induction of tumor, treated with saline solution (0.9%), named saline treated (Negative Control);

Group (III) – Control mice with induction of tumor, treated with 5-Fluoruracil (20mg/kg), named 5-Fluorouracil treated (5-FU);

Group (IV) – Group of mice with induction of tumor, treated with MCEE (400mg/kg/day, orally), named MCEE;

Group (V) – Group of mice with induction of tumor, treated with MCAE (400mg/kg/day, orally), named MCAE.

Group (VI) – Group of mice with induction of tumor, treated with MCME (400mg/kg/day, orally), named MCME.

All the groups were treated for seven days, beginning after the tumor inoculation. On the 8th day, after fasting overnight, all mice were anaesthetized with urethane (1.25g/kg) and blood samples were withdrawn by retro-orbital venipuncture technique. After, all mice were euthanized by cervical dislocation and the tumors were collected and weighed.

The blood was stored in two separate tubes: (**1**) containing the anticoagulant ethylenediamine tetraacetic acid (EDTA) and other (**2**) without anticoagulant, called dry tube (VACUETTE™, Greiner, Kremsmunster, Austria). The dry tube was immediately centrifuged (2500 *g* / 15 min) to obtain serum. Serum was utilized for determination of levels of glucose, total cholesterol, HDL-cholesterol, triglycerides, urea, creatinine, aspartate aminotransferase and alanine aminotrasferase by chemical automatic analyzer (COBAS® 6000, Roche Diagnostics, England). The tube containing EDTA was used to determine the hemathological parameters: total white blood cell (WBC) and red blood cell (RBD) were determined by standard method using haemocytometer (SYSMEX XT-4000*i*™, Sysmex Corporation, Curitiba, Brazil).

2.10 Statistical Analysis

Results were presented as the mean ± SD. The statistical analysis involving multiple comparisons were analyzed by one-way ANOVA followed by Tukey's *post hoc* test using software PRISMA (GraphPad Software, Inc., San Diego, CA, version 5.01). Values of $P < 0.05$ were considered statistically significant.

3. RESULTS

3.1 Phytochemical Constituints

The phytochemical analysis of organic extracts of *M. cauliflora* were described in the Table 1. The highest phenolic compounds in MCAE and MCME are flavonoids, phenylpropanoids, followed by proanthocyanidins. In the MCEE was detected the presence of phenolic compounds (phenylpropanoids) and steroids and triterpenes.

3.2 Anti-inflammatory Properties of Organic Extracts of M. cauliflora in the Model of Carrageenan-induced Paw Oedema

Figure 2 shows the effect of the organic extracts of *M. cauliflora* on the paw oedema induced by carrageenan in mice. Injection of carrageenan into the hind paw induced a progressive edema. The MCAE and MCME extracts (200 and 400mg/kg, orally) produced significant inhibition on inflammation induced by carrageenan in the paw oedema at both doses (MCAE: 93.5% and 83.7%, respectively; MCME: 96.7% and 95.9%, respectively). MCEE inhibited 20.3% and43.1% the inflammation at the concentration of 200 and 400 mg/kg, respectively. The treatment with ASA reduce inflammation in 73.2%).

3.3 Anti-inflammatory Properties of Organic Extracts of M. cauliflora in the Model of Carrageenan-induced Peritonitis

The induction of inflammation with carrageenan into the peritoneal cavity caused a migration of leukocytes. In a dose-dependent manner, leukocyte migration was significantly (P<0.0001) reduced in the group treated with MCAE and MCME when compared with the control group (saline), as shown in Figure 2. MCAE exerts an inhibition of 35% and 45% for treatment with 200 and 400 mg/kg, respectively. The treatment with MCME exerts an inhibition in leukocytes migration by 45% and 54% for treatment with 200 and 400 mg/kg, respectively. The treatment with 400 mg/kg of MCEE promotes a significant (P<0.05) inhibition of the 14% in the leukocytes migration. In the peritoneal fluid of the group treated with ASA (100 mg/kg) was also observed a significant (P<0.0001) decrease of the 57% in leukocyte cellular migration.

3.4 Antinociceptive Properties of Organic Extracts of M. cauliflora in the Model of Acetic Acid-induced Abdominal Writhing

The administration of MCEE showed a best reduction (P<0.0001) in the writhing induced by acetic acid administration in different concentrations (98% and 99%, for 200 and 400 mg/kg, respectively), compared with the control group treated with saline (Figure 3). The MCAE and MCME extracts (200 and 400mg/kg, orally) produced significant inhibition on writhing induced by acetic acid at both doses (MCAE: 82% and 86%, respectively; MCME: 75% and 78%, respectively). A significant decrease by 88% of writhing was observed in the group treated with ASA, the positive control for anti-nociception used in this study.

3.5 Antinociceptive Properties of Organic Extracts of M. cauliflora in the Model of Hot Plate Test

Figure 5 shows the analgesic response of the groups treated with the various organic extracts of *M. cauliflora*. The group of mice treated with a dose of 200 and 400 mg/kg of MCEE it was found a significant analgesic response (P <0.0001) compared to control (saline), in the form of increased reaction time of mice to jump or lick paws in MacDonald et al., (1946) hot plate method after 30 min. The result for the treatment with MCAE showed a significant elevation in latency time of the animals in hot plate in the administration of 200 and 400 mg/kg after 60 and 30 min, respectively. Analgesic response was not observed for the group treated with low dose of MCAE (200 mg/kg) in the time of 30 min. The treatment with MCME in both doses promotes a significant (P<0.0001) elevation in latency time of animal after 30 min. A significant increase of latency time was observed in the group treated with ASA, the positive control for anti-nociception used in this study, after 60 min of treatment.

3.6 Antioxidant Properties of Organic Extracts of M. cauliflora

All organic extracts of *M. cauliflora* had a powerful in the DPPH radical scavenging activity (Figure 1). The MCAE and MCME had the high capacity to scavenge free radicals,

3.7 Antitumoral Properties of Organic Extracts of M. cauliflora

After induction of EC solid tumor, the footpad thickness curve presented a significantly behavior from the fourth day post inoculation (Figure 6A). The saline treated group (negative control) showed significant differences in tumor growth between 6^{th} and 8^{th} days. There was a significant reduction of tumor growth in mice treated with 5-fluorouracil, MCEE, MCAE and MCME at 6^{th} and 8^{th} day, different from control group that increased the tumor weight. The EC solid tumor weight in the saline treated group was 2.31 ± 0.35g (Figure 2B). The treatment with MCEE, MCAE and MCME showed a significant antitumor activity which was further evidence by percentage reduction (30.7%, 54.5% and 44.1%, respectively). Administration of 5-fluorouracil, a commercial drug for treatment of cancer, also lowered tumor weight in 65.4%.

HDL-cholesterol, glucose and total red blood cell were significantly decreased in animals of negative control. Levels of urea, creatinine, total cholesterol and triglycerides were significantly increased in animals of negative control (Table 2). The treatment with MCEE promotes a significant improve in urea, creatinine, total cholesterol and HDL and treatment with MCME ameliorate urea, creatinine, total cholesterol and glucose levels in comparison with saline group. The 5-fluorouracil is a potent agent against tumor (Figure 1B), however, all biochemical and hematological parameters were higher in this group as adverse effects. The treatment with MCAE promoted a significant reduction in tumor mass, but did not detected significant differences in biochemical and hematological parameters when compared to the control group animal.

4. DISCUSSION

As demonstrated by the phytochemical analysis, the highest secondary compounds in organic extract tractions of *M. cauliflora* is represented by phenolic compounds in MCEE, MCAE and MCME and steroids and triterpens only in MCEE. Several studies have highlighted the presence of different phenolic compounds in fruit peels and its antioxidants potential (Hassan et al., 2011; Contreás-Caldeirón et al., 2011; Hetzroni et al., 2011; Ismail et al., 2012). The efficacy of antioxidants as a tool to treat some low to mild and chronic disorders with very low rates of progression has been widely documented in the literature (Ismail et al., 2012). Quantitative evaluation of the antioxidant activity of extracts from *M. cauliflora* suggested in the existence of substance with antioxidant acitivity. In addition, we also evaluated the acute oral toxicity of all extracts of *M. cauliflora*, which did not cause any death of mice at the dose 1000 mg/kg. *M. cauliflora*, just as other plants, contains several bioactive principles which have the potential to cause beneficial effects. These biological activities can be attributed to the presence of theses constituents.

Considering that the use of commercially analgesic and anti-inflammatory drugs exert a wide range of side effects (Vane & Botting, 1990), there is currently a strong interest in developing new therapeutic agents from plants (Iwalewa et al., 2007). The present study reported here demonstrated that organic extract fraction of *M. cauliflora* fruit peel, had significant antioxidant activity in vitro and anti-inflammatory, anti-anociceptive and antitumoral in experimental in models in mice.

We report here that the MCEE, MCAE and MCME reduced significantly the paw oedema induced by carregeenan. The carrageenam-induced inflammatory response was described in 1969 in the models of mice's paw (Levy,1969). Since the, for the development new anti-inflammatory drug this test has been more than used. According to Gemache et al. (1986), the carrageenan-induced paw oedema test controlled with the arachidonate cycloxygenase (COX) inhibitors due to its COX-dependent mechanism, thus, it is suggested that the organic extracts fractions of *M. cauliflora* has compounds that may be acting in decreased vascular permeability, mediators, such as histamine, serotonin and prostaglandins.

Cell recruitment during inflammation depends on the release of local mediators which is responsible for tissue changes as well as for the recruitment of host defense cells. These mediators are able to recruit leukocytes in the inflammation induced with carrageenan, such as neutrophilis. The tretatment with ether, acetone and methanol extracts of *M.*

cauliflora inhibited leukocyte migration induced by carrageenan (P<0.0001). This mechanism associated with this activity may be inhibition of the synthesis of inflammatory cytokines whose involvement in the cell migration is well- established. Approximately result was seen in a study with another plant, *Piptadenia stipulacea*, that a decrease on approximately 36% of leukocyte migration (Queiroz et al., 2010).

We mention here that all extracts (MCEE, MCAE and MCME) of *M. cauliflora* produced an effect significant antinociceptive in models nociception in mice. Moreover, the etheric extract showed more potent in inhibiting the acetic acid–induced nociceptive response. The acetic acid-induced has been used to confirm the peripheral anti-nociceptive activity and is considered non-specific (Chan et al., 1995), because this model test reflexes the direct interaction of the compounds with the various peripheral receptors within the peritoneal cavity (Bentley et al., 1983). The method involve the liberation of mediators, such as histamine, serotonin, cytokines and eicosanoids with an increase in peritoneal fluid that stimulate the nociceptive neurons (Zhang et al., 2005). This analgesic effect of the *M. cauliflora* could be attributed, at least in part, to its anti-inflammatory effect as, in the pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin. Santos et al. (2013) also obtained similar results to ours with inhibition of nociception of 82.3% at the dose of 400 mg / kg of aqueous extract of *Anadenanthera colubrina*.

Other test such as the hot-plate test, it's a central pain model, particularly the strong sensitivity to pain and limited tissue damage (Deraedt et al., 1980). The method induces an effect of termonociceptive skin and the integration of stimulus is due to stimulation of myelinated C fibers not driving slow (Hendry et al., 1999). The point is that the MCEE, MCAE and MCME increase the latency time of response in animal model of hot plate, and could be associated activity of supra-spinal analgesia. This effect anti-nociceptive, it has been reported with other study of the *Sutherlandia frutescens*, using aqueous extracts at doses of 5-800 mg/kg in mice (Ojewole, 2004).

Bioactive products of plants have served as a good source of antitumor treatment. Recently, several studies have been conducted and a large number of plants possessing anticancer properties have been documented (Aranjani et al., 2013; Noolu et al., 2013; Servin Wesley et al., 2013; Ramasamy et al., 2013). Administration of the MCEE, MCAE and MCME for 8 days showed an expressive and significant antitumoral activity and no adverse side effects due to the treatment were observed in biochemical (urea, creatinine,

total cholesterol, HDL-cholesterol, glucose and triglycerides) and hematological (red and white blood cells) parameters.. These results corroborate Ribeiro et al. (2012). The authors also reported the antitumor activity of aqueous and ethanolic extracts of another plant: *Arrabidaea chica* in treatment for EC tumor solid.

5. CONCLUSIONS

All organic extracts from *Myrciaria cauliflora* fruit peel presenting a significant antinociceptive and antioxidant properties. The results demonstrated that MCAE and MCME exert powerful properties against inflammation. Taking into account the above results, it can be concluded that *M. cauliflora* has significant anti-inflammatory, antinociceptive and antioxidant properties, and this study provide pharmacological evidence for a potential target for the development of new products that can be explored as alternative medicine.

CONTRIBUTORS

All authors contributed to the experimental design, data collection and manuscript writing of this study.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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Table 1. Phytochemical analysis of organic extract fractions of *M. cauliflora* fruit peel.

Compounds	MCEE	MCAE	MCME
Alkaloids (*Dragendorf)*	-	-	+
Proanthocyanins (*Vanilin Hydrochloric*)	-	++	+++
Coumarins (*UV*)	-	-	-
Flavonoids (*Neu*)	+	+++	+++
Steroids and Triterpens (*Lieberman Buchard*)	+++	-	-
Irydoids (*Vanilin Sulfuric*)	-	-	++
Phenylpropanoids (*UV*)	+++	+++	+++

(-) Non detected; **(+)** Present in low concentration; **(++)** Present in moderate concentration; **(+++)** Present in high concentration

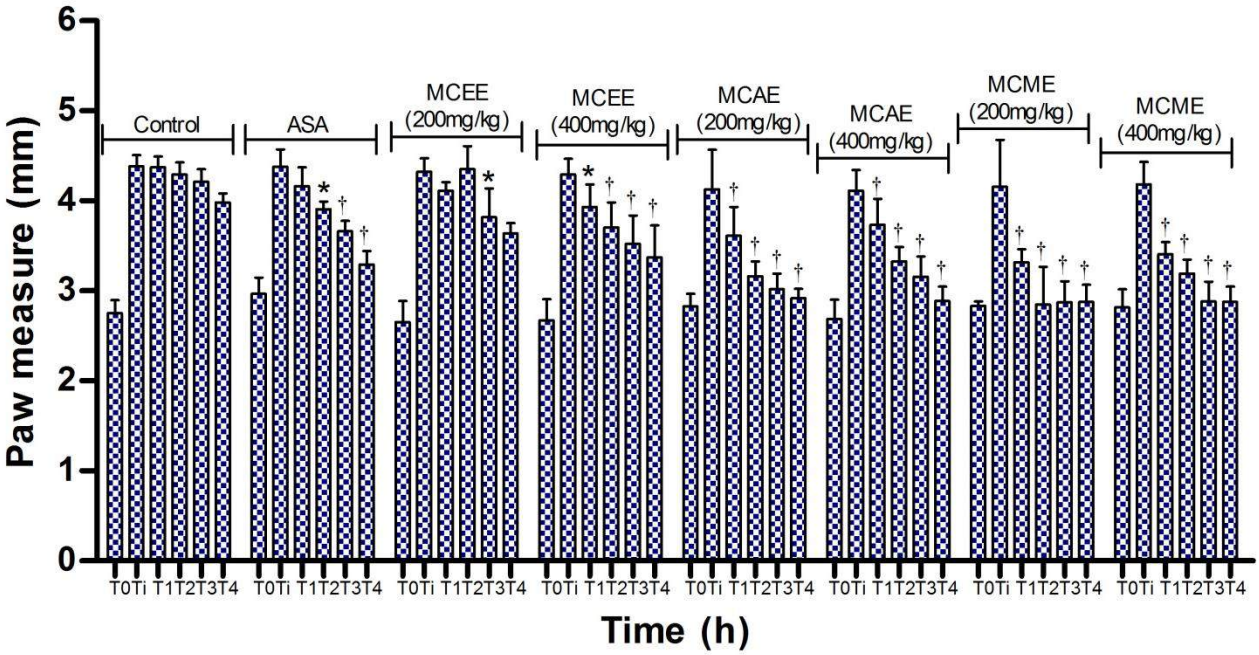


Figure 1. Effect of organic extracts of *M. cauliflora* in the thickness of the right posterior oedema of mice. ASA – Acetyl salicylicacid. MCEE – *Myrciaria cauliflora* etheric extract; MCAE – *Myrciaria cauliflora* acetonic extract; MCME – *Myrciaria cauliflora* methanolic extract. Values are expressed as mean ± S.D. **P* <0.05, in relation to control group. †*P* <0.0001, in relation to control group.

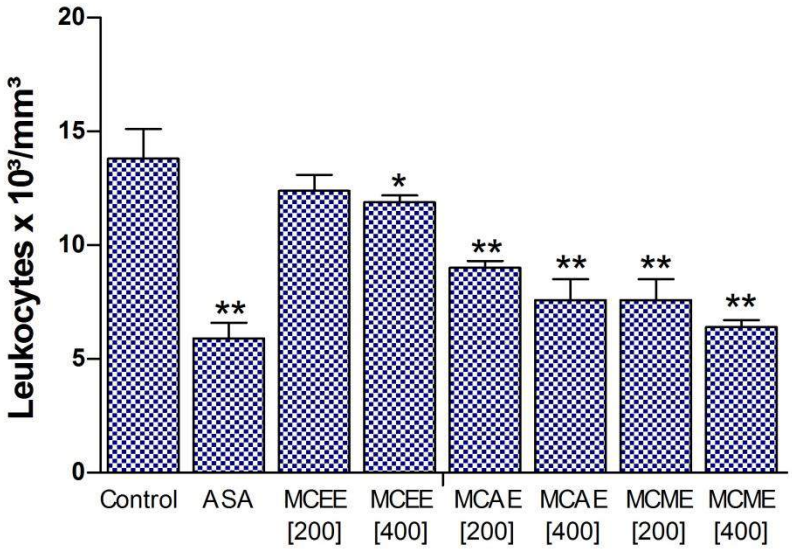


Figure 2. Effects of organic extracts of *M. cauliflora* fruit peel in the leukocyte migration in the model of carrageenan-induced peritonitis. ASA – Acetyl salicylicacid. MCEE – *Myrciaria cauliflora* etheric extract; MCAE – *Myrciaria cauliflora* acetonic extract; MCME – *Myrciaria cauliflora* methanolic extract. Values are expressed as mean ± S.D. **P* <0.05, in relation to control group. ***P* <0.0001, in relation to control group.

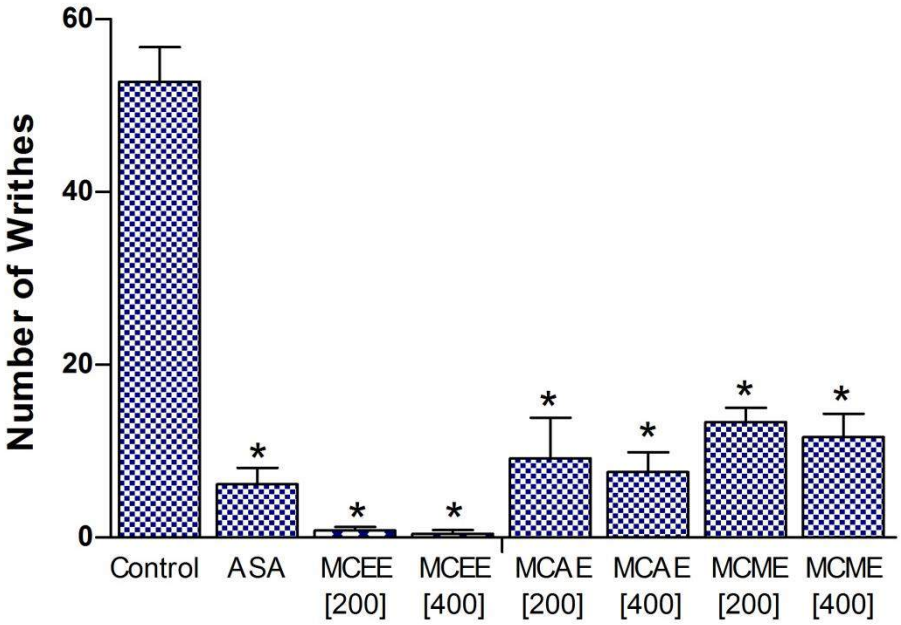


Figure 3. Effect of organic extracts of *M. cauliflora* fruit peel in the number of writhes in the model of nociception induced by acetic acid. ASA – Acetyl Salicylicacid. MCEE – *Myrciaria cauliflora* etheric extract; MCAE – *Myrciaria cauliflora* acetonic extract; MCME – *Myrciaria cauliflora* methanolic extract. Values are expressed as mean ± S.D. **P* <0.0001, in relation to control group.

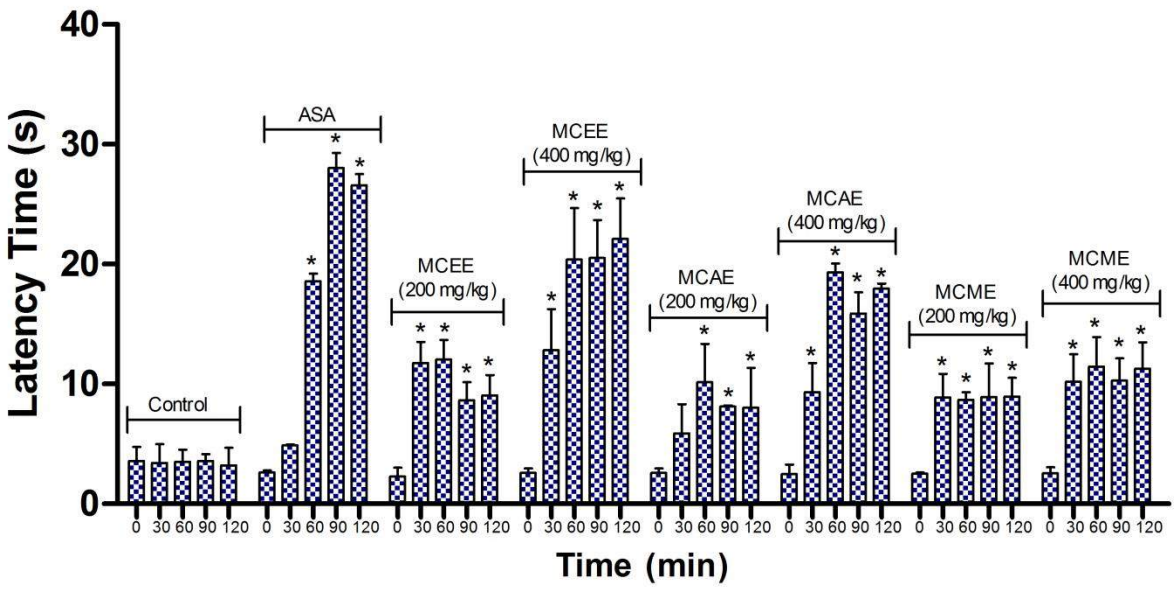


Figure 4. Effect of organic extracts of *M. cauliflora* fruit peel in the latency time in the model of nociception by hot plate. ASA – Acetyl Salicylicacid. MCEE – *Myrciaria cauliflora* etheric extract; MCAE – *Myrciaria cauliflora* acetonic extract; MCME – *Myrciaria cauliflora* methanolic extract. Values are expressed as mean ± S.D. **P* <0.0001, in relation to control group.

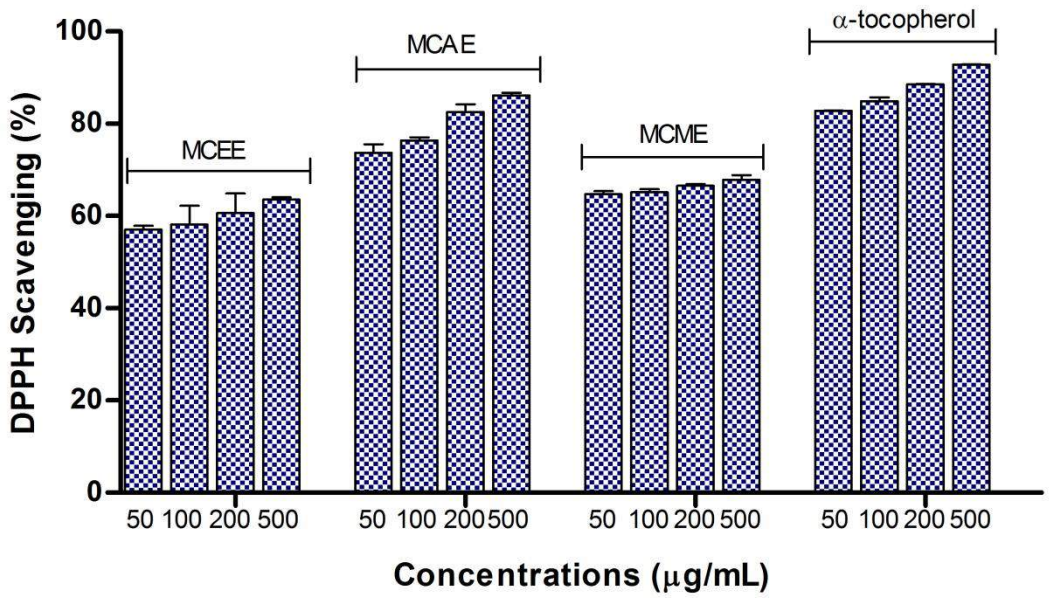


Figure 5. DPPH radical scavenging activity of organic extract of *M. cauliflora* fruit peel. DPPH - 2,2-diphenyl-1-picrylhydrazyl; MCEE – *Myrciaria cauliflora* ether extract; MCAE – *Myrciaria cauliflora* acetonic extract; MCME – *Myrciaria cauliflora* methanolic extract.

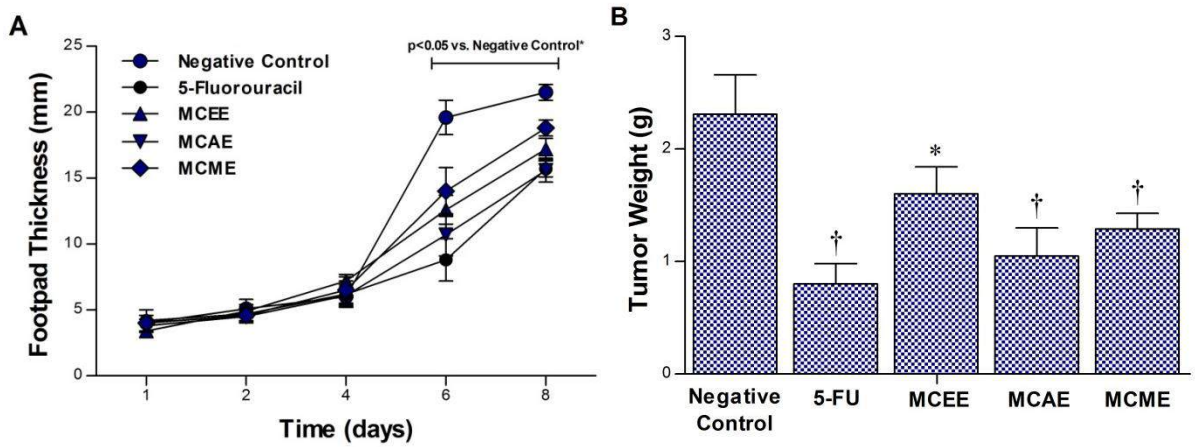


Figure 6. Effect of organic extracts of *M. cauliflora* on footpad thickness (A) and EC solid tumor weight (B). 5-FU – 5-fluorouracil; MCEE – etheric extract of *M. cauliflora*; MCAE – acetonic extract of *M. cauliflora*; MCME – methanolic extract of *M. cauliflora*. Data are reported as the mean ± S.D. Statistical differences from the controls were determined by ANOVA followed Tukey's Test. **P* <0.05 for all groups as compared to negative control. †*P* <0.0001 for all groups as compared to negative control

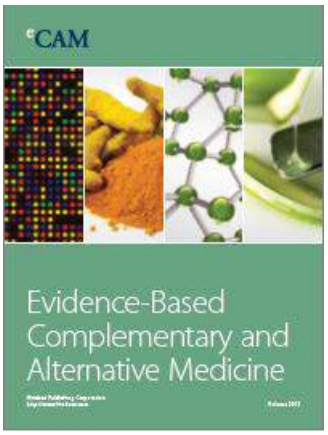
Table 2. Biological parameters in mice bearing Ehrlich solid tumor treated with different organic extracts of *M. cauliflora*.

Parameters	TREATMENT GROUP					
	Normal Control	Negative Control	5-FU	MCEE	MCAE	MCME
ALT	56.2 ± 7.60	63.0 ± 2.3	517.4 ± 69.1**,††	56.6 ± 17.8	46.6 ± 10.7	51.0 ± 13.2
AST	115.0 ± 6.8	115.8 ± 18.6	601.0 ± 64.9**,††	90.6 ± 16.4	107.5 ± 55.4	77.4 ± 13.2
Urea	27.6 ± 3.78	46.55 ± 5.68**	48.67± 5.07**	34.6 ± 2.2††	28.2 ± 1.2††	33.1 ± 0.7††
Creatinine	0.21 ± 0.01	0.50 ± 0.06**	0.65 ± 0.09**	0.18 ± 0.01††	0.15 ± 0.01††	0.13 ± 0.05††
Glucose	116.1 ± 10.4	81.3 ± 2.04**	79.5 ± 2.8**	88.3 ± 5.7**	117.40 ± 6.2††	119.40 ± 19.6††
Total cholesterol	120.6 ± 9.1	146.1 ± 10.4*	178.1 ± 11.3**,†	111.2 ± 11.8††	114.7 ± 15.3†	102.1 ± 15.4††
HDL-cholesterol	69.1 ± 5.2	43.3 ± 3.1**	42.1 ± 8.2**	54.6 ± 2.9††	60.5 ± 3.1††	51.4 ± 4.9
Triglycerides	118.7 ± 5.9	292.5 ± 29.2**	209.4 ± 25.1**,†	310.5 ± 40.8**	111.8 ± 27.6††	227.7 ± 30.5**
Total RBC	98.0 ± 6.9	81.2 ± 8.6*	71.2 ± 8.6*	75.2 ± 3.8	96.1 ± 4.3 ††	84.0 ± 5.6
Total WBC	1.31 ± 0.05	1.25 ± 0.09	0.35 ± 0.06**,††	1.21 ± 0.02	1.24 ± 0.01	1.26 ± 0.01

AST – aspartate aminotransferase; ALT – alanine aminotransferase; RBC – red blood cells (x10^5 cells/mL); WBC – white blood cells (x10^4 cells/mL); *P <0.05, in relation to normal control group. **P <0.0001, in relation to normal control. †P <0.05, in relation to negative control. ††P <0.0001, in relation to negative control group.

VIII. ARTIGO 4

"Lectin from *Crataeva tapia* Bark Improves Tissue Damages and Plasma Hyperglycemia in Alloxan-Induced Diabetic Mice"



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Research Article

Lectin from *Crataeva tapia* Bark Improves Tissue Damages and Plasma Hyperglycemia in Alloxan-Induced Diabetic Mice

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Crataeva tapia is a plant popularly used for diabetes treatment, in Brazil. Progressive decline in renal and hepatic functions has been described in patients with diabetes mellitus, and mortality rate is increased in patients with chronic liver and renal disease. This study aimed to evaluate whether *Crataeva tapia* bark lectin (CrataBL) improves hyperglycemia and renal and hepatic damage in diabetic mice. CrataBL was purified by ion exchange chromatography on CM-cellulose, and intraperitoneal administration of CrataBL to alloxan-induced diabetic mice at dose of 10 mg/Kg/day and 20 mg/Kg/day for 10 days significantly reduced serum glucose levels by 14.9% and 55.9%, respectively. Serum urea, creatinine, aspartate aminotransferase, and alanine aminotransferase were also significantly reduced after treatment with both doses of CrataBL. Furthermore, histological analysis of liver, kidney, and pancreas revealed an improvement in the tissue morphology upon treatment with CrataBL. The results suggest that CrataBL has a beneficial hypoglycemic activity and improves the renal and hepatic complications of diabetes. Therefore, this lectin may be a promising agent for the treatment of diabetes, and this might be the basis for its use in the folk medicine as an alternative treatment to manage diabetes-related complications such as hyperglycemia and tissue damage.

1. Introduction

Crataeva tapia (also known as *Crateva tapia*), a plant of Capparidaceae family, is commonly found in Pluvial Tropical Atlantic Forest and Pantanal Tropical Forest in Brazil [1]. *C. tapia* is known by Northeast Brazilian people as "paudalho" or "tapiá" and its bark is largely used in the folk medicine for the treatment of diabetes. Recently, a lectin with a molecular weight of 40 kDa (CrataBL) was purified from the aqueous extract of *Crataeva tapia* bark [2]. Lectins are carbohydrate binding proteins, of nonimmunogenic origin, that bind specifically and reversibly to different types of carbohydrates or glycoproteins and can be obtained from several sources, mainly from vegetal [3]. Several plant lectins have been demonstrated to possess a variety of biological activities including antitumor [4–6], anti-inflammatory [7, 8], antimicrobial [9–11], analgesic [4], antioxidant [3] insecticidal [2, 12–14], anticoagulant [15], and hypoglycemic [16, 17].

Diabetes mellitus is a chronic disease considered to be one of the five leading causes of death in the world, and it is a complex metabolic disease with great development of pathological changes in many tissues [18]. The

disease is characterized by alteration in the carbohydrate metabolism resulting in an increase of the glucose levels [19]. Approximately 360 million of adult people have diabetes, corresponding to 8.3% of the world with diabetes, and this is projected to rise to 552 million by 2030, corresponding to 9.9% of the world population [20]. The hyperglycemia in diabetes produces superoxide anions, which generate hydroxyl radicals, promoting cell membrane damages as a result of lipid peroxidation and protein glycation of membrane [18]. In diabetic individuals the major alterations occur in renal and hepatic tissue and have been associated with functional and morphological damage in these organs [21, 22]. Among the common complications of diabetes the nephropathy is a chronic disease that affects 40% of individuals. Diabetic nephropathy is responsible for 50% of chronic renal failure cases [23]. Furthermore, hepatic dysfunction promoted by diabetes can result in nonalcoholic steatosis, hepatomegaly amongst others [24].

Studies have reported that the doubts about the efficacy and safety of some of the oral hypoglycemic agents have prompted a search for safer and more effective drugs in the treatment of diabetes [25]. Thus, the aim of the present study was to investigate whether CrataBL from *C. tapia* bark is a metabolite with potential antihyperglycemic activity.

2. Material and Methods

2.1. Chemicals. Alloxan monohydrated and CM-cellulose was purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, USA. Insulin (Humolin N) was purchased from Lilly, Brazil. All the other chemicals used were in an analytical grade.

2.2. Plant Material. C. tapia barks were collected from the Recife City, PE, Northeast Brazil. The plant was identified by *Instituto Agronômico de Pernambuco* (IPA) and a voucher specimen was deposited (n° 61.415).

2.3. Purification of Crataeva Tapia Bark Lectin. C. tapia bark lectin was obtained through a sequential purification protocol as previously reported by Araújo et al. [2]. Powdered bark (10 g) was suspended in 0.15 M NaCl (100 mL). After homogenization in a magnetic stirrer (16 h at 4°C), followed by filtration through gauze and centrifugation (4,000 ×g, 15 min), the supernatant (crude extract) was taken as starting material. Soluble proteins in crude extract were fractionated with ammonium sulphate and the 30–60% precipitate fraction (30–60 F) was submitted to dialysis (3,500 Da cut-off membrane, 4°C) against distilled water (2 h) followed by 10 mM citrate-phosphate buffer pH 5.5 (2 h). The 30–60 F was applied (11 mg of protein, hemagglutinating activity of 1024) into a CM-cellulose chromatography column (5.2 cm × 1.6 cm) equilibrated with 10 mM citrate-phosphate buffer pH 5.5 at flow rate of 20 mLh^{-1}. The unabsorbed proteins were eluted with the buffer solution until the absorbance at 280 nm was lower than 0.05; CrataBL was eluted with 0.5 M NaCl. Protein concentration was determined according to Lowry et al. [26] using bovine serum albumin as standard.

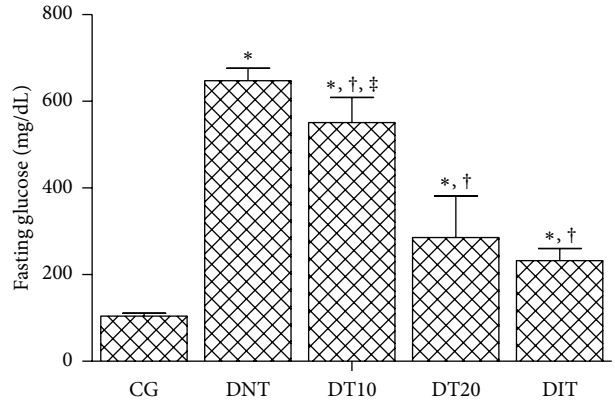

Figure 1: Fasting serum glucose levels in diabetic mice after treatment with CrataBL. CG: control group; DNT: diabetic nontreated; DT10: diabetic treated with CrataBL (10 mg/kg); DT20: diabetic treated with CrataBL (20 mg/kg); DIT: diabetic treated with insulin (10 mg/kg). $^{*}P < 0.05$ versus CG; $^{\dagger}P < 0.05$ versus DNT; $^{\ddagger}P < 0.05$ versus DIT.

2.4. Animals. Female albino Swiss mice (*Mus musculus*), six weeks of age, weighing 30 ± 5 g, bred in the Biotherium of *Departamento de Antibióticos*, UFPE, Brazil, were housed in colony cages (six mice per cage) at room temperature of 22 ± 2°C, relative humidity 40–60%, and 12 h light and 12 h dark cycle. The mice were fed standard rodent diet (Labina, Purina Brazil Ltd., Brazil) and water *ad libitum*. The experimental protocol was approved by the Animal Care and Use Committee at the Federal University of Pernambuco, Brazil (CEEA-UFPE-Ofício n° 40/06). All experimental procedures were conducted in accordance with the ethical guidelines for Care and Use of Laboratory Animals.

2.5. Induction of Diabetes in Mice. Experimental diabetes was induced in overnight-fasted mice by a single intraperitoneal injection of freshly prepared alloxan monohydrated (80 mg/kg in 0.9% NaCl solution). After alloxan administration, all animals were relocated to their cages and given free access to food and water. Diabetes was confirmed by measuring the fasting blood glucose levels 72 h after alloxan injection. The mice with serum glucose of >250 mg/dL were considered diabetic and were included in the study.

2.6. Experimental Design. The mice were split into four groups ($n = 6$, for group) as follows:

Group (I)—normoglicemic mice receiving saline solution (0.9%), as control group;

Group (II)—diabetic control mice, named diabetic nontreated;

Group (III)—diabetic mice treated with CrataBL (10 mg/kg/day, intraperitoneally) in saline solution (0.9%) for 10 days, named diabetic treated 10;

Group (IV)—diabetic mice treated with CrataBL (20 mg/kg/day, intraperitoneally) in saline solution (0.9%) for 10 days, named diabetic treated 20;

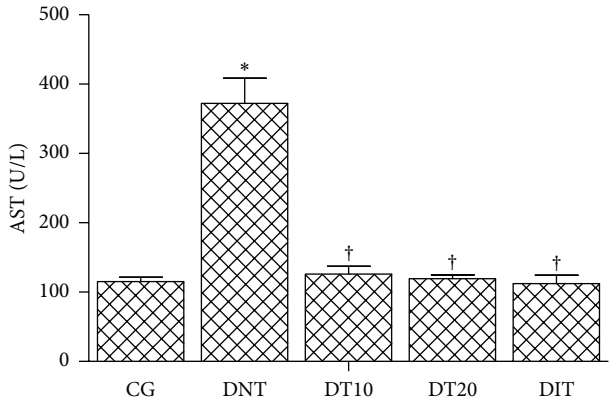

Figure 2: Serum aspartate aminotransferase levels in diabetic mice after treatment with CrataBL. CG: control group; DNT: diabetic nontreated; DT10: diabetic treated with CrataBL (10 mg/kg); DT20: diabetic treated with CrataBL (20 mg/kg); DIT: diabetic treated with insulin (10 mg/kg). $^{*}P < 0.05$ versus CG; $^{\dagger}P < 0.05$ versus DNT.

Group (V)—diabetic mice treated with insulin (10 mg/kg/day, intraperitoneally) for 10 days, named diabetic insulin treated.

Before and at the end of the experimental period, overnight fasting mice were anaesthetized with 2% xylazine hydrochloride (10 mg/kg) and 10% ketamine hydrochloride (115 mg/kg); blood samples were withdrawn with a capillary from mice-cavernous sinus for biochemical parameters determination [27]. The mice were sacrificed by cervical dislocation. Thereafter, pancreas, liver, and kidneys were excised and immediately fixed in 10% neutral buffered formalin for histological analysis.

2.7. Effect of CrataBL on Biochemical Data. Mice blood samples were centrifuged at 2,500 g for 15 min at 4°C (Sorvall RC6, NC, US). Sera were obtained and the levels of the glucose, urea, creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured by enzymatic colorimetric methods (Labtest Diagnostica, Brazil/SA) in a chemistry autoanalyzer (COBAS 6000, Roche Diagnostics, England).

2.8. Histological Analysis of Pancreas, Kidneys, and Liver. Pancreas, kidney, and liver from all groups were subjected to standard routine tissue processing technique: dehydrated in gradual ethanol (50–100%), cleared in xylene, and embedded in paraffin. Sections of 5 μm thickness were cut from each block and stained with haematoxylin-eosin for histological examination. Prepared slides were studied by light microscopy and all sections were evaluated for the tissue injury.

2.9. Statistical Analysis. Values were expressed as the mean ± SD. Multiple comparisons were analyzed by one-way ANOVA followed by Tukey's *post hoc* test. For all analysis the 0.05 level of probability was used as the criterion of significance. The analyses were carried out using software PRISMA (GraphPad Software, Inc., San Diego, CA, version 5.01).

Table 1: Serum urea and creatinine levels in diabetic mice after treatment with CrataBL.

Groups	Urea	Creatinine
CG	34.3 ± 6.8	0.30 ± 0.01
DNT	58.9 ± 5.8*	0.39 ± 0.04*
DT10	46.7 ± 6.6*,†	0.33 ± 0.05†
DT20	44.0 ± 2.9*,†	0.32 ± 0.04†
DIT	43.1 ± 2.6†	0.32 ± 0.02†

CG: control group; DNT: diabetic nontreated; DT10: diabetic treated with CrataBL (10 mg/kg); DT20: diabetic treated with CrataBL (20 mg/kg); DIT: diabetic treated with insulin (10 mg/kg). $^{*}P < 0.05$ versus CG; $^{\dagger}P < 0.05$ versus DNT.

3. Results and Discussion

3.1. Effect of CrataBL on Fasting Glucose. Diabetes is a complex metabolic disorder with a characteristic modulation of glucose metabolism. Chronic hyperglycemia promotes tissue damage which can be found in many organs and systems, with consequent often serious disease [28]. Alloxan, a prominent diabetogenic chemical with ability to generate reactive oxygen species formation that induce death of β cell of the pancreas by necrosis [29], is considered a good model for reproducible induction of the metabolic state of this disease in experimental animals [30–33]. Thus, in this study the mice subjected to alloxan injection showed symptoms of severe diabetes such as hyperglycemia. Insulin treatment, as a positive control, validates our model by showing the improvement in diabetes.

In a previous study, the acute toxicity of CrataBL was determined in mice; at the doses from 300 mg/kg to 2,000 mg/kg, mice did not present weight loss or death, and LD_{50} of CrataBL was 2,500 mg/kg [4]. Therefore, CrataBL concentrations used in the present study are considered safe, without problem of toxicity, and indicate that the lectin is a potential pharmaceutical substance.

As demonstrated in Figure 1, CrataBL proved to be an effective hypoglycemic agent after 10 days of treatment and showed significant antihyperglycemic activity in a dose-dependent manner, in alloxan-induced diabetic mice, and at the dose of 20 mg/kg/day it exhibited better glucose reduction (56%) than 10 mg/kg/day (15%), and it was similar to that found by the treatment with the standard drug, insulin (64%), without no significant difference ($P > 0.05$). Studies with soya bean lectin reported a decrease of 17.3% in blood glucose, and it was suggested that this effect is due to an increase in pancreatic growth stimulated by the lectin [34]. Wang et al. [35] demonstrated that *Agaricus bisporus* lectin administration could partially reverse the impaired β-cell growth potential by regulating cell cycle proteins (cyclin D1, cyclin D2, and Cdk4). So, induction of pancreatic β-cell proliferation by lectins suggests the therapeutic potential in decreasing blood glucose and treating experimental diabetes mellitus [34, 35].

Medicinal plants are gaining wide acceptably worldwide because they are the potential sources of bioactive agents in use as pharmaceutics. In a fast changing world, a number of procedures to evaluate hypoglycemia as well as the kidney

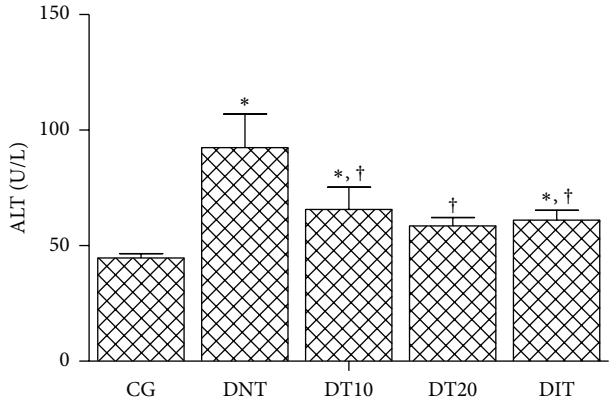

Figure 3: Serum alanine aminotransferase levels in diabetic mice after treatment with CrataBL. CG: control group; DNT: diabetic nontreated; DT10: diabetic treated with CrataBL (10 mg/kg); DT20: diabetic treated with CrataBL (20 mg/kg); DIT: diabetic treated with insulin (10 mg/kg). $^{*}P < 0.05$ versus CG; $^{\dagger}P < 0.05$ versus DNT.

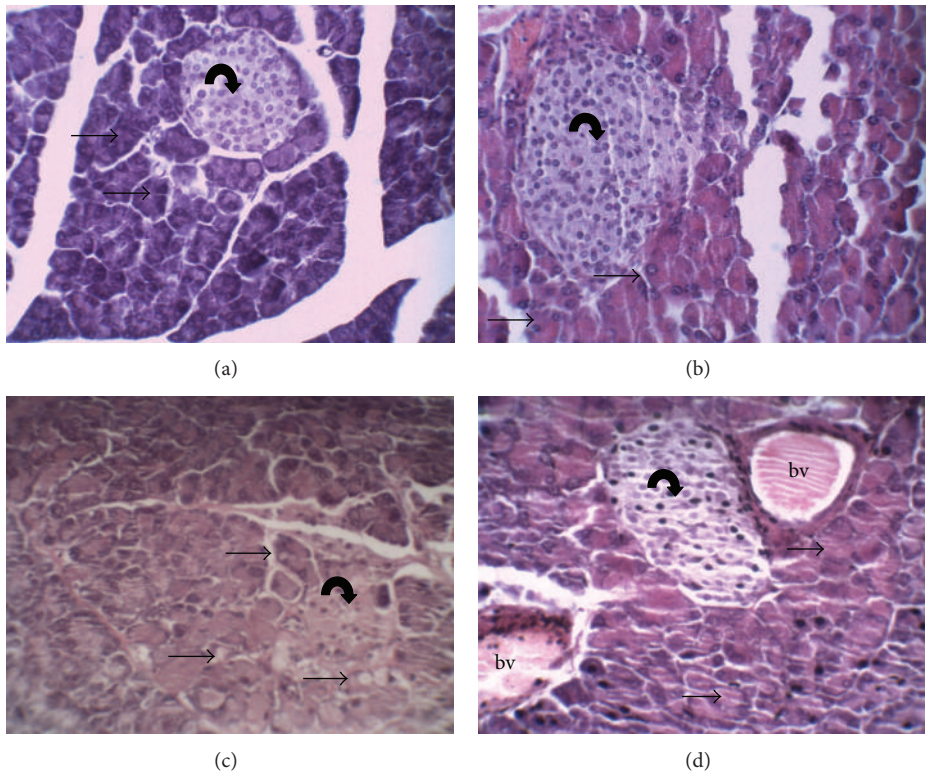

Figure 4: Histopathological changes in pancreatic tissue. (a) Control group—preserved pancreatic islet of Langerhans (curved arrow) and preserved pancreatic acinar cells (straight arrows); (b) diabetic nontreated—atrophic pancreatic islet of Langerhans with few cells (curved arrow) and the presence of some vacuoles in the pancreatic acinar cells (straight arrows); (c) diabetic treated with CrataBL (10 mg/kg)—pancreatic islet of Langerhans (curved arrow) and preserved pancreatic acinar cells (straight arrows); (d) diabetic treated with CrataBL (20 mg/kg)—preserved pancreatic islet of Langerhans (curved arrow) and preserved pancreatic acinar cells (straight arrows) and blood vessel (bd). Haematoxylin-eosin: 400x.

and liver damage have been used to investigate the effectivity of new natural agents which are explored by experts and clinicians [36–39].

3.2. Effects of CrataBL on Markers of Kidney Damage. As shown in Table 1, levels of urea and creatinine known as kidney function markers were significantly increased in sera of alloxan-induced diabetic mice, in comparison with normal mice. After 10 days of treatment with CrataBL, the levels of urea and creatinine significantly decreased. The diabetic mice treated with CrataBL at doses of 10 and 20 mg/kg reduced serum levels of urea by 20.7% and 25.3%, respectively, and the same doses decreased creatinine concentration by 15.4% and 17.9%, respectively. Insulin, the positive control for treatment,

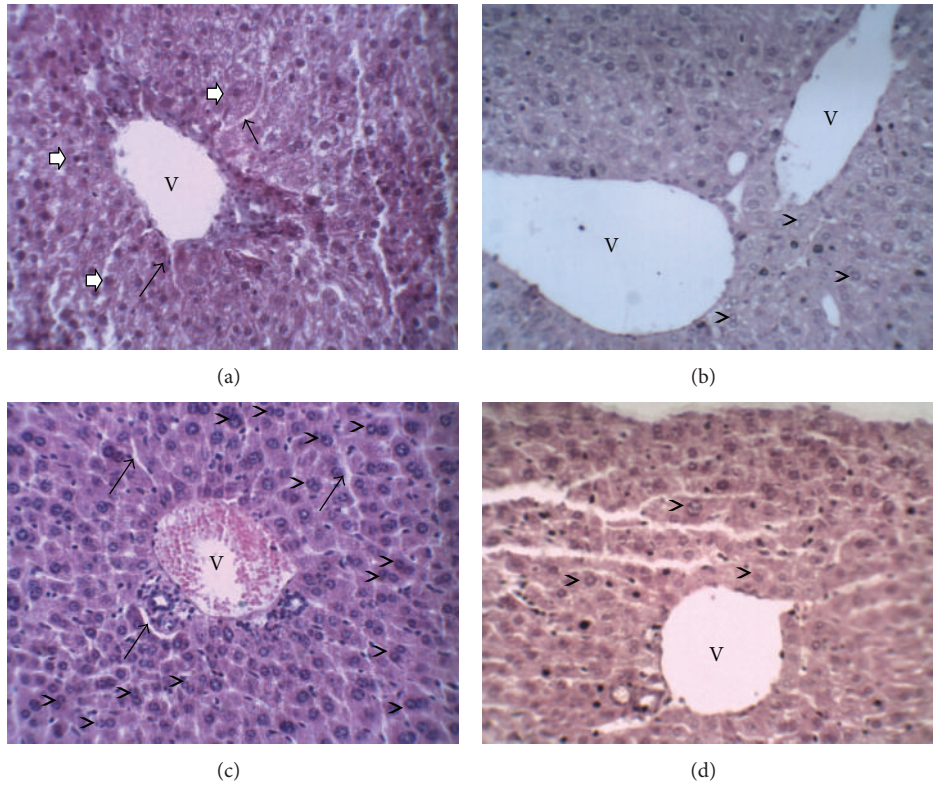

Figure 5: Histopathological changes in hepatic tissue. (a) Control group—centrilobular vein (V), preserved hepatocytes (white arrows), and sinusoidal capillaries (thin arrows); (b) diabetic nontreated—centrilobular vein with many red blood cells (V), intense mitotic activity in hepatocytes (arrowheads), and the presence of sinusoidal capillaries (thin arrows); (c) diabetic treated with CrataBL (10 mg/kg)—centrilobular vein (V) and considerable mitotic activity in hepatocytes (arrowheads); (d) diabetic treated with CrataBL (20 mg/kg)—centrilobular vein (V) and preserved hepatocytes (arrowheads). Haematoxylin-eosin: 400x.

decreased these markers of renal damage by 26.8% and 17.9%. Our results are in agreement with recent reports by Kumar et al. [39], Omara et al. [40], and Yankuzo et al. [41] who demonstrated that renal damage can be ameliorated when the levels of serum urea and creatinine are decreased by treatment with extracts of medicinal plants.

Kidney damage is usually associated with diabetes. In the initial course of disease the presence of hypertrophy of the glomeruli and tubular cells, matrix expansion, and enhanced renal blood flow is common, and these alterations have been postulated to cause loss of renal function [40, 41]. High levels of urea and creatinine are usually reported as one of the most sensitive markers of kidney damage, and it is reported that renal hypertrophy in diabetic animals is caused by an increased formation of advanced glycation end products and accumulation of glycogen granules in distal tubules [42, 43].

Thus, our results clearly indicate that CrataBL possesses an effective potential to improve kidney damage induced by alloxan-diabetes.

3.3. Effects of CrataBL on Markers of Liver Damage. As compared to the control groups, the activities of the markers of liver damage serum AST and ALT were significantly ($P < 0.05$) reduced in alloxan-induced diabetic mice after treatment with 10 or 20 mg/kg of CrataBL; the activity of AST was reduced by 66.2% and 67.9%, respectively (Figure 2) and ALT activity was decreased by 28.9% and 36.6%, respectively (Figure 3). These percentages of reduction were similar to those observed with insulin treatment. Therefore, administration of CrataBL for 10 days reversed the elevated levels of liver marker enzymes, which reflects the capability to conserve the membrane integrity of cellular and mitochondrial membranes of hepatocyte in alloxan-diabetic mice treated with this lectin.

Our results are in agreement with those of Mansour et al. [25] who reported that hepatic damage can be improved by decreasing the levels of serum AST and ALT in alloxan-induced diabetic rats subjected to treatment with herbal bioactive agents. It is well known that liver is the focal organ of oxidative and detoxifying processes [22]. Liver diseases are a high problem of health worldwide and the release of intracellular localized marker enzymes such as AST and ALT into the blood when cell and mitochondria are subjected to injury indicates hepatocytes damage [44, 45]. Furthermore, the elevated serum levels of AST and ALT in nontreated diabetic mice (Figures 2 and 3) indicate that alloxan caused liver damage and loss of the functional integrity of the hepatocyte membranes, as also evidenced in a study reported by Rajesh and Latha [45] about hepatotoxicity of polyherbal formulation.

As indicated by serum levels of AST and ALT CrataBL is able to improve liver damage.

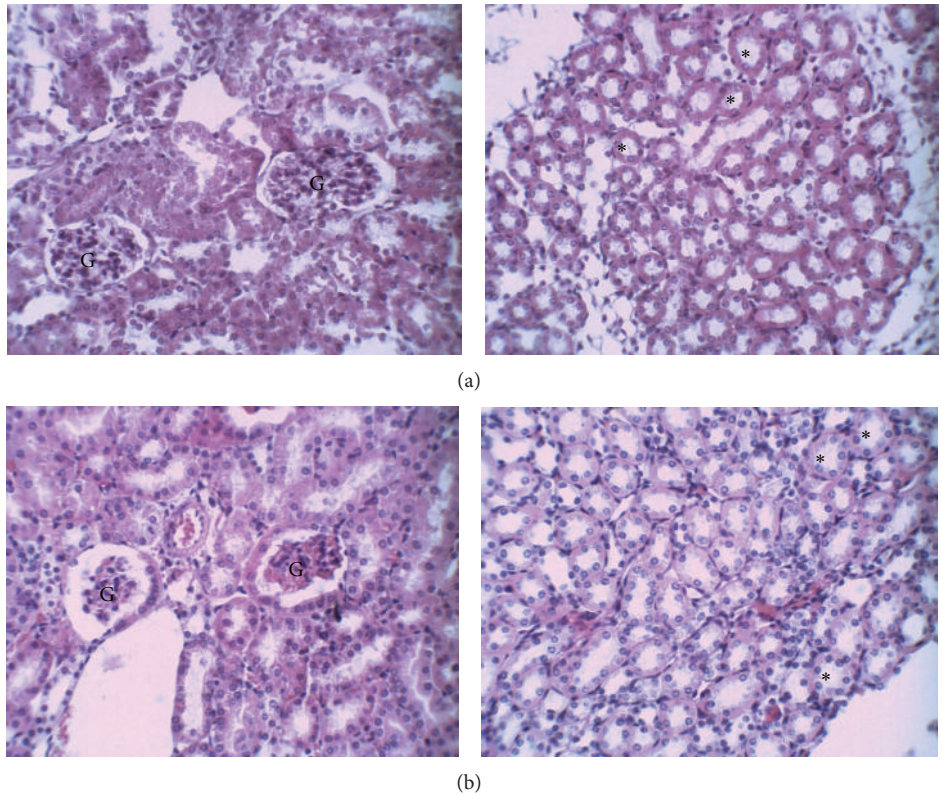

Figure 6: Histopathological changes in renal tissue of the normal and diabetic mice. (a) Control group—renal glomeruli (G) with preserved subcapsular spaces (left) and collecting tubules (stars) without changes in the medullary region (right); (b) diabetic nontreated—retracted glomerular tufts (G) with increased subcapsular space and evident thickening of parietal layer of Bowman's capsule due to have been entirely replaced by the cuboidal cells (left) and preserved collecting tubules (stars) (right). Haematoxylin-eosin: 400x.

3.4. Effects of CrataBL on the Histopathological Changes of the Pancreas, Liver, and Kidneys. The structure of the pancreas of the control and diabetic mice are shown in Figure 4. Pancreas of control group showed normal pancreatic islet of Langerhans and acinar cells (Figure 4(a)). By contrast, in alloxan-induced diabetic mice the acinar cells were altered with presence of vacuoles; furthermore deterioration of pancreatic islets was also observed (Figure 4(b)). CrataBL (10 mg/kg) treatment increased the number of pancreatic islets as compared to that of diabetic nontreated animals (Figure 4(c)). Interestingly, pancreatic section of diabetic mice treated with CrataBL (20 mg/kg) showed pancreatic islet similar to that of the control group (Figure 4(d)).

The histopathological analysis of pancreas isolated from mice administrated with alloxan alone revealed tissue damage with deterioration of pancreatic islets. In this connection, it may be observed that several authors reported such changes in pancreas tissues of mice exposed to prominent diabetogenic alloxan for its ability to induce reactive oxygen species (ROS) formation, resulting in the selective necrosis of beta cells in pancreatic islets [29, 39, 46, 47]. However, the diabetic animals treated with lectin from *C. tapia* bark showed normal architecture of pancreatic tissue, suggesting the regeneration of pancreatic islet by CrataBL administrations. The ability of lectins to stimulate pancreatic growth has been reported [48]. The regenerative action of CrataBL corroborates with *Agaricus bisporus* lectins (ABL). The ABL administration could partially reverse the impaired β-cell growth potential by induction of pancreatic islet proliferation [35]. Thus, the antidiabetic effect observed by CrataBL administration suggests the therapeutic potential in preventing and/or treating diabetes.

Figure 5 shows the photomicrographs of hepatic tissues of control group and diabetic experimental groups. The section of liver tissue of control mice demonstrates preserved hepatocytes, centrilobular vein, and sinusoidal capillaries (Figure 5(a)). In the alloxan-induced diabetic mice the histopathological analysis of hepatic tissue shows intense mitotic activity in hepatocytes (Figure 5(b)). CrataBL (10 mg/kg) treatment exhibited considerable mitotic activity in hepatocytes (Figure 5(c)). Similar to the control group, diabetic mice treated with CrataBL (20 mg/kg) also revealed an equivalent architecture of hepatic tissue (Figure 5(d)).

The photomicrographs of renal tissues are represented in Figures 6 and 7. Figures 6(a) and 6(b) represent the renal tissues of control group and diabetic nontreated group, respectively. Kidneys of control group show normal architecture of tissue with preserved subcapsular spaces in glomeruli and collecting tubules without change in the medullary region. Differently, the renal tissue of alloxan-induced diabetic mice shows retracted glomerular tufts with increased subcapsular space and evident thickening of Bowman's membrane due

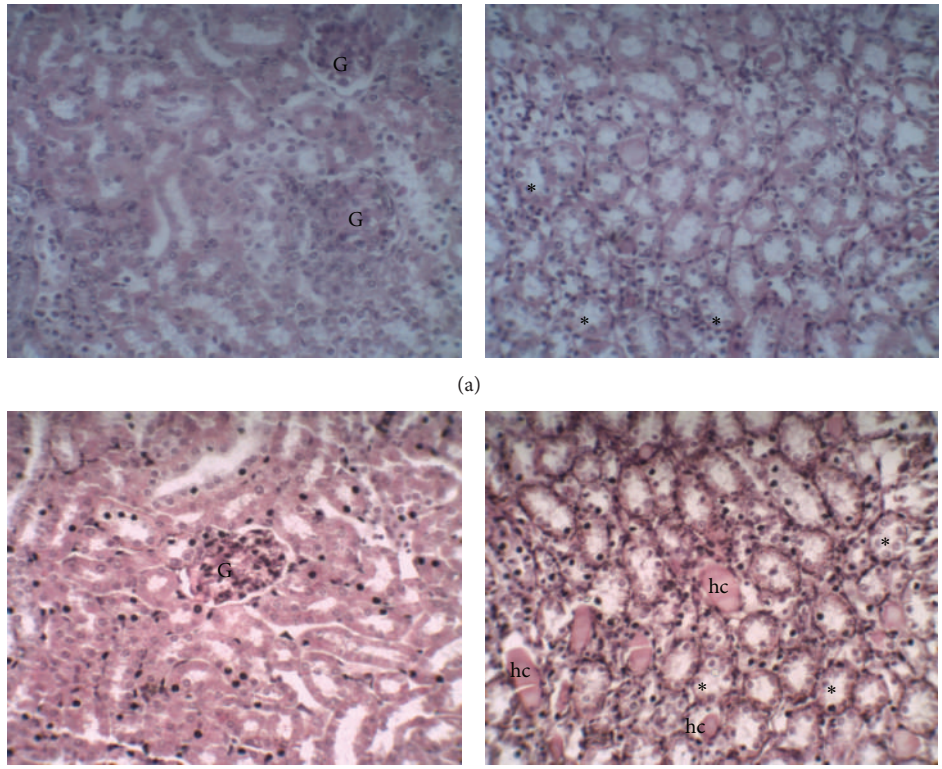

Figure 7: Histopathological changes in renal tissue of the diabetic mice treated with CrataBL. (a) Diabetic treated with CrataBL (10 mg/kg)—renal glomeruli (G) with irregular subcapsular spaces and some distinctly collapsed (left) and collecting tubules with slight swelling of the tubular epithelium (stars) (right); (b) diabetic treated with CrataBL (20 mg/kg)—preserved renal glomeruli (G) (left) and presence in the medullary region of collecting tubules (stars) with evident swelling of the tubular epithelium and hyaline casts (hc) (right). Haematoxylin-eosin: 400x.

to the cuboid appearance of epithelial cells. In kidneys of alloxan-induced mice treated with CrataBL (10 mg/kg) renal glomeruli were evident with irregular subcapsular spaces and some distinctly collapsed (Figure 7(a)). However, renal sections of diabetic mice treated with CrataBL (20 mg/kg) show preserved renal glomeruli and presence in the medullary region of collecting tubules with evident swelling of the tubular epithelium and hyaline casts presence (Figure 7(b)).

The elevated levels of glucose contribute to the generation of ROS in the diabetes, which promotes to the increase of oxidative stress in various organs and tissues [49, 50]. In addition, the hyperglycemia provokes hepatic and renal damage and consequently has been associated with histological and functional alterations and liver and kidneys [51, 52]. In fact, these organs are the focal of important organic functions and damage promoted by diabetes can result in severe complications with nephropathy and nonalcoholic steatosis [23, 24]. The current study demonstrated that CrataBL treatment improves the hepatic and renal histologic damage induced by diabetes. These findings correlated with improved biochemical markers of liver and renal functions by CrataBL. Taken together, these results may contribute to a better understanding of the regenerative effect of CrataBL in pancreas and protective in liver and kidneys, emphasizing the utilization of this lectin in the treatment of complications associated with diabetes mellitus.

4. Conclusion

Our results indicate that CrataBL is a good agent in controlling diabetes induced by alloxan and improves the damage on kidneys and liver tissues. The findings of this study also indicate that the active principle present in *C. tapia* is CrataBL, which is a lectin responsible for the antihyperglycemic activity found in this study and that could explain the basis for its use in the folk medicine as an alternative treatment for diabetes. Therefore, we conclude that CrataBL serves as an excellent candidate for an alternative therapy in the treatment of diabetes mellitus since it revealed an antidiabetic activity and other beneficial effects that ameliorate diabetes and associated complications.

Conflict of Interests

The authors declare that they have no conflict of interests.

Acknowledgments

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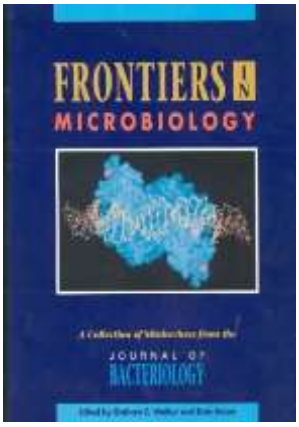
IX. ARTIGO 5

"Organic extracts from *Indigofera suffruticosa* leaves have antimicrobial and synergic actions with erythromycin against *Staphylococcus aureus*"



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Organic extracts from *Indigofera suffruticosa* leaves have antimicrobial and synergic actions with erythromycin against *Staphylococcus aureus*

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A characteristic feature of *Staphylococcus aureus* is its ability to acquire resistance to antimicrobial agents. There is a need, therefore, for new approaches to combat this pathogen; for example, employing a combination of plant-derived products and antibiotics to overcome bacterial resistance. *Indigofera suffruticosa* is a plant popularly used to treat infections and has verified antimicrobial action. Here, we investigate the antimicrobial activity of different extracts from *I. suffruticosa* against *S. aureus* and their synergistic effects with erythromycin. Leaves of *I. suffruticosa* were extracted sequentially using diethyl ether, chloroform and acetone and the antimicrobial activity of each extract then tested against nine clinical isolates of *S. aureus*. Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined by microdilution tests, while the fractional inhibitory concentration (FIC) was assessed by checkerboard assay. All organic solvent extracts showed antimicrobial activity against *S. aureus* strains. The acetone extract was the most potent inhibitor of *S. aureus* (MIC and MBC of 0.78 and 3.12 mg/mL), followed by the chloroform extract (MIC and MBC of 3.12 and 6.25 mg/mL). Furthermore, acetone or chloroform extracts of *I. suffruticosa* enhanced the activity of erythromycin against *S. aureus* (FIC $\leq$ 0.5). We conclude that organic extracts from leaves of *I. suffruticosa*, alone or combined with erythromycin, are promising natural products for the development of new anti-*S. aureus* formulations.

Keywords: plant extracts, antibacterial agents, macrolide antibiotic, *S. aureus*

INTRODUCTION

Patients in hospital intensive care units are at risk of acquiring nosocomial infections due to the use of invasive devices and/or extended hospital stay (Streit et al., 2004). Long-term hospitalization may further complicate patient health by exposure to various antimicrobial agents. Additionally, the indiscriminate use of antibiotics in treating infections promotes bacterial evolution and emergence of resistance strains (Palmer and Kishony, 2013; Tavares et al., 2013). *Staphylococcus aureus* is an important pathogen associated with nosocomial human infections, and this microorganism has successfully evolved numerous strategies to resist different antibiotics (Coutinho et al., 2009; Chung et al., 2011). Such increases in antibiotic resistant *S. aureus* strains drives research discovery of new antimicrobial agents and the development of alternative therapeutic strategies. These include plant extracts, which have considerable antimicrobial potential (Leite et al., 2006; da Silva et al., 2013; Zakavi et al., 2013).

Medicinal plants are important health and economic components used by many cultures for thousands of years (Agra et al., 2008; Silva et al., 2012). According to the World Health Organization approximately 80% of the global population uses medicinal plants or herbal medicine for primary health care (Pereira et al., 2012). Brazil has the highest plant diversity of any country and represents 20% of biodiversity in the world. *Indigofera suffruticosa* Mill (Fabaceae, Papilionidae) is a plant originally from Antilles and Central America, popularly known as "anileira" or "anil," and was introduced into Brazil for the extraction of indigo, a blue dye blue (Indigo Blue) widely used by the textile industry. Although some toxic effects are reported for this plant, such as hemolytic anemia and hemoglobinuria in

Table 1 | Pharmacological potential of *Indigofera suffruticosa*.

Scientific account	Related compounds
Gastroprotective agent acute ulcer stimulating prostaglandin, mucus and HSP70. (Luiz-Ferreira et al., 2011)	Ethyl acetate fraction from methanolic extract showed the best action and the authors highlighted the role of role of flavonoids and alkaloids presents in AcF as active compounds
In vivo action against *Pediculosis capitis* (García Calixto et al., 2011)	An effective treatment using 5% *I. suffruticosa* Mill tincture was reported in a patient infested with *Pediculosis capitis*
Immunostimulatory and antitumoral actvities *in vitro* (Lopes et al., 2011)	This study evaluated the action of both alkaloid fraction and pure indigo. Indigo showed high activity which suggest that it is the major active principle in *I. suffruticosa*
Antimycobacterial (Carli et al., 2010)	These authors did not isolate or detected any compounds. The methanolic extract showed better activity than dichloromethane
Anticonvulsant effect (Almeida et al., 2013)	Alkaloids, flavonoids, steroids, proteins, carbohydrates, indigo carmine and essential oils (Linalool and Pinene) were detected in the methanolic extract
Anti-inflammatory property *in vivo* (Chen et al., 2013a)	Eight phenolic compounds were quantified: salicylic acid, syringic acid (major compounds) ρ-coumaric acid, vanillin, syringaldehyde, quercetin, isoliquiritigenin, formononetin. Salicylic acid was found in the plasma of mice fed with *I. suffruticosa* extracts
In vivo increase of Phase II detoxification enzyme and glutathione levels (Chen et al., 2013b)	The authors reported the same compounds quantified by Chen et al. (2013a). Ethanolic extracts showed the best action on the induction of phase II detoxification enzyme, and syringic acid was the most active among phenolic compounds detected, however, it was less potent than ethanolic extracts

cattle and guinea pig (Salvador et al., 2011), it has been used in traditional medicine both externally and internally (Barros and Teixeira, 2008). Moreover, pharmacological effects of *I. suffruticosa* have been confirmed scientifically, such as anti-inflammatory (Chen et al., 2013a), anticonvulant (Almeida et al., 2013) and wound healing (Luiz-Ferreira et al., 2011) (**Table 1**). Previous work by our group has shown that aqueous infusions of *I. suffruticosa* leaves have inhibitory activity against *S. aureus* and dermatophyte strains (fungi) (Leite et al., 2006), though their action against clinical isolates and synergic potential have yet to be studied.

Synergistic assessments have become a key tool in phytomedicine research in recent years, and uses of antibiotics in combination with herbal products have been investigated as antimicrobials for *S. aureus* resistant strains (Wagner and Ulrich-Merzenich, 2009). Some studies have used erythromycin, a 14-membered ring macrolide antibiotic and therefore part of the Macrolide-Lincosamide-Streptogramin-B (MLSB) family, as a representative drug to evaluate combinatory effects of plant-derived products (Chan et al., 2013, 2015). Antibiotics from the MLSB family serve as an important combatant against *S. aureus* methicillin resistant (MRSA) strains, which are a major cause of disease in the general population and hospital-acquired infections (Pantosti, 2012). MLSB comprises three unrelated groups (macrolide, lincosamide and streptogramin-B) that share the same binding site in bacterial ribosome. It is possible, therefore, that a synergistic effect for one group might predict a similar action from the other groups.

Given this background, our study aimed to define the antimicrobial activities of different organic extracts from *I. suffruticosa* leaves against *S. aureus* strains (MRSA and MSSA), and then to examine synergistic actions with erythromycin.

MATERIALS AND METHODS

CHEMICALS

Dimethylsulfoxide (DMSO), erythromycin and 7-hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt (Resazurin) was purchased from Sigma-Aldrich Chemical Company, St. Louis, MO, while Mueller-Hinton Agar and Nutrient Agar medium were from HIMEDIA Laboratories®. Diethylether, chloroform and acetone were obtained from Merck, Darmstadt, Germany.

PLANT MATERIAL AND PREPARATION OF ORGANIC EXTRACTS

Leaves of *I. suffruticosa* were collected in São Caetano, Pernambuco, Brazil (latitude: 08° 19′ 33″ S; longitude: 36° 04′ 21″ W) between 10 and 11 a.m. The plant was identified by Dr. Marlene Carvalho Alencar Barbosa (Department of Botany, UFPE) and a voucher specimen deposited at the UFP Geraldo Mariz Herbarium-UFPE (identification number 45.217).

Organic extracts were prepared by successively extracting dried leaves of *I. suffruticosa* (100 g) with 200 mL of diethyl ether, chloroform or acetone, common solvents arranged in order of increasing polarity. Briefly, the leaf powder was homogenized firstly with 200 mL of diethyl ether for 2 h in a mechanical stirrer, kept refrigerated overnight (4°C) and filtered with Whatman

no.1 paper. The solvent was then removed under reduced pressure in a rotary evaporator at 45°C to produce diethyl ether extract. The plant material which was not extracted by diethyl ether was then homogenized with 200 mL chloroform and all extraction process was repeated generating the chloroform extract. Finally, the remaining powder was submitted to acetone extraction to produce acetone extract. All dried organic extracts of *I. suffruticosa* were stored at −20°C until use and dissolved in dimethyl sulfoxide (DMSO, 1%) before each test.

PHYTOCHEMICAL SCREENING

An approximate amount of diethyl ether, chloroform and acetone extracts from *I. suffruticosa* leaves were subjected to phytochemical analysis to ascertain the presence of secondary metabolites such as alkaloids, flavonoids, phenylpropanoids, triterpenoids and volatile oil in according to Wagner and Bladt (2009). Briefly, compounds classes were visualized as aid thin layer chromatography (TLC) on silicagel 60 F254 (Merck), mobile phase standard and Dragendorff, NEU-PEG, KOH-Ethanol, Liebermann-Burchard and vanillin-sulfuric acid reagents, respectively. Tests for tannins, saponins and other heterosides were not performed due to the low polarity of the extracts.

ANTIMICROBIAL ASSAYS

Staphylococcus aureus strains

The antimicrobial activity was tested against the following microorganisms provided by the Departamento de Antibióticos, Universidade Federal de Pernambuco (UFPEDA): *Staphylococcus aureus* (UFPEDA 02), and some isolated strains of *S. aureus* originally obtained from: vaginal secretion (UFPEDA 660); catheter tip (UFPEDA 663); urine sample (UFPEDA 670); blood sample (UFPEDA 672); prostate secretion (UFPEDA 676); wound secretion (UFPEDA 677 and 679); ocular secretion (UFPEDA 687). Strains UFPEDA 670 and 672 are classified as MRSA strains (**Table 2**). All strains were and maintained in Mueller-Hinton Agar (MHA) and stored at 4°C.

Table 2 | Susceptibility to antibiotics of *Staphylococcus aureus* strains[a].

UFPEDA	Source	Susceptibility to antibiotics			
		Oxacillin	Cefoxitin	Erythromycin	Clindamycin
02	ATCC 6538	S	S	S	S
660	Vaginal secretion	S	S	S	S
663	Catheter tip	S	S	S	S
670[b]	Urine sample	R	R	R	R
672[b]	Blood sample	R	R	R	R
676	Prostate secretion	S	S	S	S
677	Wound secretion	S	R	R	S
679	Wound secretion	S	S	R	S
687	Ocular secretion	S	S	S	S

R, resistant; S, sensitive. [a]Data provided by UFPEDA Collection. [b]MRSA.

Determination of antibacterial activity using the disc diffusion method

The antibacterial activity of the organic extracts of *I. suffruticosa* leaves was determined by the disc diffusion method (de Oliveira et al., 2012). Briefly, all clinically isolated *S. aureus* strains were grown on MHA medium at 37°C for 18 h, suspended in distilled water (approximately 1.5×10^8 CFU/mL) and 100 μL aliquots of bacterial suspension were immediately inoculated in Petri dishes containing MHA medium. Sterile paper discs (6 mm diameter) containing 20 μL organic extracts of *I. suffruticosa* (100 mg/mL) were applied to the agar and the Petri dishes incubated at 37°C for an additional 18 h. Following incubation, the diameter of the inhibition zone (DIZ) of growth was measured, using DMSO as negative control.

Effects of temperature and pH on antimicrobial activity

The antimicrobial activity of each *I. suffruticosa* extract against *S. aureus* UFPEDA 02 was determined. Samples were placed in sterile tubes and kept for 30 min at different temperatures (28, 30, 60, and 100°C), or were stored at a different pH for 30 min at 25°C, using 1M NaOH or 1M HCl to adjust the pH range between 3 and 10. The antibacterial activity of treated extracts was tested using the disc diffusion method, as described above.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The minimal inhibitory concentration (MIC) was determined by a microdilution broth susceptibility assay (Clinical and Laboratory Standards Institute, 2011). Two-fold serial dilutions of the organic extracts of *I. suffruticosa* containing 50–0.20 mg/mL in DMSO were prepared in Mueller-Hinton Broth (MHB; 200 μL) in a 96-well microtiter plate. Bacterial suspensions were prepared from each *S. aureus* strains freshly grown in Mueller-Hinton broth (Merck) (approximately 1.5×10^8 CFU/mL,) and 10 μL of this suspension was added to each well. After incubation at 37°C for 24 h, bacterial growth was recorded using a Resazurin solution (0.01%). MIC was the lowest concentration at which no color change (from purple to pink) was observed. Afterwards, cultures were seeded in MHA medium and incubated for 24 h at 37°C to determine the minimum bactericidal concentration (MBC), which corresponds to the lowest amount of extract that kills *S. aureus*. All experiments were performed in triplicate.

Evaluation of combinatory effects of extracts and erythromycin

Combinatory effects between organic extracts of *I. suffruticosa* and erythromycin were assessed using the checkerboard test against the strain UFPEDA 02. Briefly, samples with different proportions of plant extract:drug (final volume: 20 μL) were prepared from stock solutions of each extract (50 mg/mL) and erythromycin (1 mg/mL) and antibacterial activity was tested as described for MIC determination (da Silva et al., 2013). The Fractional Inhibitory Concentration (ΣFIC) was calculated according to the equation:

$$\Sigma\text{FIC} = (\text{MICE} + \text{D}/\text{MICE}) + (\text{MICD} + \text{E}/\text{MICD})$$

MICE+D: minimal inhibitory concentration of extract in combination with erythromycin; MICD+E: minimal inhibitory concentration of erythromycin in combination with extract. Results were considered: synergistic (ΣFIC < 0.5); additive ($0.5 <$ ΣFIC < 1); non-interactive ($1 <$ ΣFIC < 4); or antagonist (ΣFIC > 4) (Vuuren and Viljoen, 2011).

STATISTICAL ANALYSIS

Each experiment was performed in triplicate and results are expressed as the mean ± standard deviation (SD). Statistical analyses were performed by ANOVA and unpaired Student's *t*-test. All analyses were carried out using software StatView, version 4.5, Abacus Concept, Inc, Berkeley, CA. Differences were considered significant at $p < 0.05$. The correlation indices were calculated using the Pearson coefficient (ρ).

RESULTS

PHYTOCHEMICAL ANALYSIS

TLC analysis revealed the presence of flavonoids, phenylpropanoids, triterpenoids and volatile oils in all three extracts.

Table 3 | Phytochemical analysis of organic extract from leaves of *Indigofera suffruticosa*.

Compounds class	*Indigofera suffruticosa* extracts		
	Ether	Chloroform	Acetone
Alkaloids	-	+	-
Flavonoids	+	+	++
Phenylpropanoids	++	+	+
Triterpenoids	+	+	+
Volatile oils	+	++	+

(-) absent, (+) weak, (++) strong.

Table 4 | Antimicrobial activity of organic extracts from leaves of *Indigofera suffruticosa* against *Staphylococcus aureus* strains.

S. aureus strains	Organic extracts of leaves of *Indigofera suffruticosa* DIZ		
	Ether	Chloroform	Acetone
02	$34.7 \pm 0.6^{a,1}$	$36.0 \pm 0.0^{a,1}$	$35.7 \pm 1.1^{a,1}$
660	$29.0 \pm 1.7^{b,1}$	$28.0 \pm 2.0^{b,1}$	$28.0 \pm 2.0^{b,1}$
663	$28.7 \pm 0.6^{b,1}$	$27.7 \pm 0.6^{b,1}$	$26.7 \pm 0.6^{b,1}$
670	$32.7 \pm 1.1^{a,1}$	$27.7 \pm 2.5^{b,2}$	$30.7 \pm 0.6^{b,2}$
672	$32.6 \pm 1.1^{a,1}$	$32.3 \pm 0.6^{c,1}$	$31.0 \pm 3.0^{b,1}$
676	$27.3 \pm 0.6^{b,1}$	$25.3 \pm 0.6^{b,1}$	$26.3 \pm 0.6^{b,1}$
677	$30.0 \pm 1.0^{b,1}$	$29.0 \pm 1.7^{b,1}$	$29.7 \pm 0.6^{b,1}$
679	$29.0 \pm 1.0^{b,1}$	$26.3 \pm 2.3^{b,1}$	$25.7 \pm 2.1^{b,1}$
687	$26.7 \pm 2.3^{b,1}$	$26.0 \pm 2.6^{b,1}$	$25.3 \pm 2.1^{b,1}$
Average DIZ	30.08 ± 2.7	28.7 ± 3.4	28.78 ± 3.4

DIZ values are expressed in mm.

**Same superscript letter (^{a,b,c}) indicates no significant difference ($p > 0.05$) between DIZ values from different strains for each solvent (same column).*

***Same superscript number (^{1,2}) indicates no significant difference ($p > 0.05$) between DIZ values from different solvents against each strain (same row).*

In most of the tests performed, only quantitative differences were found. Thus, flavonoids, phenylpropanoids and volatile oils predominated in acetone, ether and chloroform extracts, respectively. Alkaloids or nitrogen-containing compounds were detected only in the chloroform extract of *I. suffruticosa* (**Table 3**).

ANTIBACTERIAL ACTIVITY OF ORGANIC EXTRACTS FROM LEAVES OF *I. SUFFRUTICOSA*

All organic extracts of leaves of *I. suffruticosa* showed antimicrobial activity against different *S. aureus* strains. However, the inhibition varied according to the extract and test strain with DIZ values ranging from 25.3 ± 2.1 to 36.0 ± 1.0 mm (**Table 4**). All extracts were active against both MRSA strains (UFPEDA 670 and UFPEDA 672) with DIZ values >30.0 mm, except for the chloroform extract which gave a DIZ of 27.7 ± 2.5 mm for strain UFPEDA 670. Diethyl ether extracts showed the best inhibition (30.08 ± 2.69 mm), followed by acetone (28.79 ± 3.35 mm) and chloroform (28.7 ± 3.42 mm), however no significant differences were observed between these average DIZ values ($p > 0.05$). Furthermore, strong correlations were found between the DIZ of all extracts with ρ-values of 0.86, 0.94, and 0.92 for ethyl/chloroform, and chloroform/acetone ethyl/acetone extracts, respectively. The antimicrobial activity of the extracts was not affected ($p > 0.05$) after high temperature treatment (**Figure 1A**) or variation of pH (**Figure 1B**), except for the ether extract which was notably more active at pH 8 ($p > 0.05$).

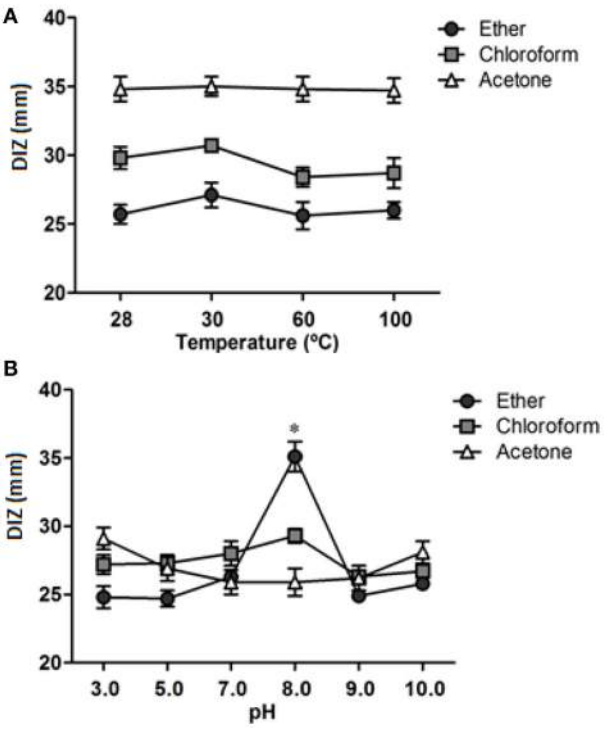

FIGURE 1 | Stability of organic extracts of leaves of *Indigofera suffruticosa*. (A) Effect of temperature on the stability of organic extracts of *I. suffruticosa*. **(B)** Effect of pH on the stability of organic extracts of *I. suffruticosa*. DIZ—inhibition zone diameter. *Significant differences in relation to control.

Table 5 | Minimum inhibitory concentration and minimum bactericidal concentration of organic extracts from leaves of *Indigofera suffruticosa* against *Staphylococcus aureus* strains.

***S. aureus* strains**	**Organic extracts from leaves of *Indigofera suffruticosa***								
	Ether			**Chloroform**			**Acetone**		
	MIC	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**	**MIC**	**MBC**	**MBC/MIC**
02	3.12	12.5	4	3.12	12.5	4	1.56	3.12	2
660	6.25	12.5	2	6.25	25.0	4	1.56	6.25	4
663	6.25	25.0	4	6.25	25.0	4	3.12	12.5	4
670	6.25	25.0	4	6.25	25.0	4	1.56	12.5	8
672	6.25	12.5	2	6.25	12.5	2	3.12	6.25	2
676	6.25	12.5	2	3.12	12.5	4	3.12	3.12	1
677	6.25	25.0	4	3.12	6.25	2	3.12	6.25	2
679	6.25	12.5	2	3.12	6.25	2	1.56	6.25	4
687	6.25	12.5	2	6.25	12.5	2	0.78	12.5	16
MIC_{50}	6.25			6.25			1.56		
MBC_{50}	12.5			12.5			6.25		
Average MIC	5.9 ± 1.0			4.85 ± 1.6			2.16 ± 0.9		
Average MBC	16.67 ± 6.2			15.27 ± 7.7			7.63 ± 3.8		

MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration.

MIC_{50}, concentration able to inhibit 50% of strains; MBC_{50}, concentration able to kill 50% of strains.

MIC, MIC_{50}, MBC and MBC_{50} are expressed in mg/mL.

The MIC and MBC values ranged from 0.78 to 6.25 mg/mL and 3.12 to 25.0 mg/mL, respectively, with the acetone extract having the lowest values (**Table 5**). The MIC_{50} (minimum concentration able to inhibit 50% of strains) was 1.56 mg/mL for the acetone extract, and 6.25 mg/ml for both ether and chloroform extracts. Similarly, the MBC_{50} (minimum concentration able to kill 50% of strains), for the acetone extract was 6.25 mg/mL, but 12.5 mg/mL for ether and chloroform extracts. Additionally, the average MIC and MBC of acetone extract (2.16 ± 0.9 and 7.63 ± 3.8, respectively) were lower ($p > 0.05$) than other extracts (4.85 ± 1.6 and 15.27 ± 7.7 for ether extract; and 5.9 ± 1.0 mg/mL and 16.67 ± 6.2 mg/mL for chloroform). The three extracts also differed in their MBC/MIC ratio (Pankey and Sabath, 2004); although ether and chloroform extracts showed exclusively bactericidal effects (MBC/MIC ratios ranged from 2 to 4), the acetone extract had both bactericidal and bacteriostatic actions, however this extract was a bactericidal agent for almost all *S. aureus* strains tested (77.78%).

Table 6 | Combinatory effects of organic extracts from leaves of *Indigofera suffruticosa* and erythromycin against *S. aureus* UFPEDA 02.

Erythromycin/ Extracts proportion	**Organic extracts from *Indigofera suffruticosa* leaves (ΣFIC)**		
	Ether	**Chloroform**	**Acetone**
9:1	0.9	0.9	0.4
8:2	0.9	0.4	0.4
7:3	0.7	0.7	0.3
6:4	0.6	0.3	0.6
5:5	0.6	0.2	0.5
4:6	0.8	0.8	0.8
3:7	1.2	0.3	0.3
2:8	0.8	0.8	0.8
1:9	0.8	1.7	1.7
Average ΣFIC	0.81 ± 0.18	0.68 ± 0.46	0.644 ± 0.44

COMBINATORY EFFECTS OF ORGANIC EXTRACTS OF *I. SUFFRUTICOSA* AND ERYTHROMYCIN

When the antimicrobial actions of erythromycin and *I. suffruticosa* organic extracts were tested in combination, additive, synergistic and non-interactive actions were observed (**Table 6**); importantly, no antagonistic effects were noted. Acetone extract and erythromycin showed synergistic effects (in five ratios (55.56%; ΣFIC values ranged from 0.3 to 0.5), additive effects ($0.6 \leq \Sigma FIC \leq 0.8$) in three and a non-interactive effect in only one (ratio of 1:9, drug:extract; $\Sigma FIC = 1.7$). For the chloroform extract and erythromycin combinations both synergistic ($0.2 \leq \Sigma FIC \leq 0.4$) and additive ($0.7 \leq \Sigma FIC \leq 0.9$) effects were equally found in four ratios and only one ratio gave a non-interaction (1:9, drug:extract; $\Sigma FIC = 1.7$). No synergistic effect was seen with ether extracts, but 8 ratios resulted in additive effects ($0.6 \leq \Sigma FIC \leq 0.9$) and 1 ratio a non-interactive effect (3:7, drug:extract; $\Sigma FIC = 1.2$). Strong correlations were observed between ΣFIC values from erythromycin/acetone and erythromycin/chloroform combinations ($\rho = 0.82$), although no significant difference was found between the mean of their ΣFIC values (0.68 ± 0.46 and 0.644 ± 0.44; $p < 0.05$). The best ΣFIC values were 0.2 for erythromycin/chloroform at 5:5, followed by 0.3 for all these combinations: erythromycin/acetone (at 7:3 and 3:7) and for erythromycin/chloroform (at 3:7 and 6:4).

DISCUSSION

S. aureus is a pathogen long-recognized to be capable of developing drug resistance which increases patient treatment time, rate of morbidity and mortality, and associated financial costs (Pantosti, 2012). These factors make the search for new active agents against *S. aureus* highly relevant. In contrast to the well-known antimicrobial effects of *I. suffruticosa* (Leite et al., 2006; Carli et al., 2010), our present work is the first to evaluate organic solvent extracts for activity against clinical isolates of *S. aureus* strains (including two MRSA strains), as well their combinatory effects with a macrolide drug (erythromycin).

The organic extracts from *I. suffruticosa* leaves showed antimicrobial activity against all tested strains of *S. aureus* and, importantly, high inhibition zones were found against MRSA strains (UFPEDA 670 and UFPEDA 672). These two strains were isolated from different sources and exhibited multidrug-resistant profile (oxacillin-cefoxitin-erythromycin-clindamycin). The best anti-*S. aureus* activity was shown by the acetone extract, since its MIC_{50} was 4-fold lower than the MIC_{50} values of the two other extracts. From chemical point of view, the acetone extract contains more flavonoids than ether and chloroform extracts. It is known that different species of genus *Indigofera* including *I. suffruticosa* are rich source of bioactive flavonoids (Hasan et al., 1993; Narender et al., 2006; Varanda et al., 2011; Perez et al., 2013). Previous chemical analysis from *I. suffruticosa* resulted in the identification of four quercetin derivatives. Although our result revealed that the antimicrobial properties might be associated with the presence of flavonoids, a characterization of acetone extract is necessary, even though this has not been our major focus.

We also showed that high temperature (up to 100°C) had negligible effect on the anti-*S. aureus* activity of each extract, which may explain the effective traditional usage of *I. suffruticosa* in infusions prepared by prolonged boiling of its leaves (Corrêa, 1984). Similarly, the antimicrobial activities of our three organic extracts showed little change when submitted to pH values ranging from pH 3 to pH 10. Thermal and pH stabilities are noteworthy factors for development of new antimicrobial formulations by the cosmetic, food and pharmaceutical industries, and our findings encourage further research into use of our organic extracts.

Exploring combinatory effects of antimicrobial agents and natural products is an attractive strategy to overcome bacterial resistance (Betoni et al., 2006; Wink et al., 2012). Diverse targets are involved in the synergistic effects of drugs and plant-derived products such as enzymes and substrates, metabolites, receptors, ion channels, transport proteins, DNA and RNA (Wagner, 2011; Yang et al., 2014). Our study establishes that all organic extracts from *I. suffruticosa* induce at least additive effects with erythromycin. In addition to its more potent antimicrobial activity, the synergestic effect of the acetone extract was higher than that of the chloroform extract, although this did not reach statistical significance and the ΣFIC values of the two were strongly correlated. In contrast, the *I. suffruticosa* ether extract only showed additive effects or, in one tested ratio, a non-interactive effect. These results suggest these as a promising source of potential compounds to be used in combination of erythromycin (and other members of MLSB family).

I. suffruticosa extracts have been target of a various studies in order to prove their medicinal potential. Most of these works have shown that polar solvent extracts are more active (**Table 1**) as they are rich in phenolic compounds, flavonoids, carbohydrates, glycoproteins, indigo, alkaloids, and triterpenes (Leite et al., 2006; Carli et al., 2010; Lopes et al., 2011; Luiz-Ferreira et al., 2011; Almeida et al., 2013; Chen et al., 2013a,b). Furthermore, extracts from *I. suffruticosa* have been also shown key features to be used as a medicine such as lethal dose 50% (1600 mg/kg (ip) in mice (Almeida et al., 2013) and induction of phase II detoxification enzyme and increase of glutathione levels in rat Clone 9 liver cells (Chen et al., 2013b).

In summary this paper showed that organic extracts of *I. suffruticosa* are promising natural products for the development of new anti-*S. aureus* formulation given their antimicrobial inhibiting MRSA strains and their combination with erythromycin seems to be very perspective, thus deserving further studies in order to understand their mechanism of action.

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8. CONCLUSÕES

- Hiperuricemia está bastante relacionada com o fenótipo CHTG em homens do Nordeste do Brasil e pode aumentar em muito o risco de morte por evento cardiovascular em dez anos nessa população;
- Obesidade abdominal e hipertrigliceridemia, isolados ou associados, constituindo o fenótipo cintura hipertrigliceridêmica, são distúrbios metabólicos bastante frequentes e apresentam uma relação fisiopatológica importante com hiperuricemia em homens não-diabéticos da Região Nordeste do Brasil;
- Os extratos fracionados obtidos da casca do fruto maduro de *M. cauliflora* apresentam grande quantidade de compostos fenólicos e não são capazes de promover toxicidade oral aguda em camundongos;
- Epicarpo de frutos maduros de *M. cauliflora* demonstrou ter um grande potencial terapêutico para hiperuricemia e os processos de inflamação, dor, estresse oxidativo e câncer;
- As substâncias presentes no extrato acetônico do epicarpo de frutos de *M. cauliflora*, bem como lectina de *C. tapia*, demonstraram ser agentes promissores para o tratamento da diabetes e das complicações bioquímicas e danos teciduais causados por esse distúrbio metabólico;
- Os extratos orgânicos das folhas de *I. suffruticosa*, sozinhos ou em combinação com eritromicina, apresentaram-se como produtos naturais promissores para o desenvolvimento de novas formulações farmacêuticas contra *S. aureus*.

ANEXO 1

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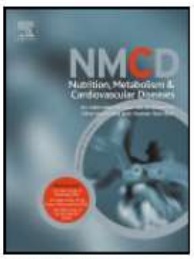

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