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AVALIAÇÃO DA ATIVIDADE ANTI-HIPERGLICEMIANTE DE EXTRATOS DE *Parkinsonia aculeata* EM RATOS DIABÉTICOS

Ana Catarina Rezende Leite

Orientadora: Profa. Dra. Vera Lúcia de Menezes Lima
Co-orientadora: Profa. Dra. Maria Bernadete Maia

Recife, 2006

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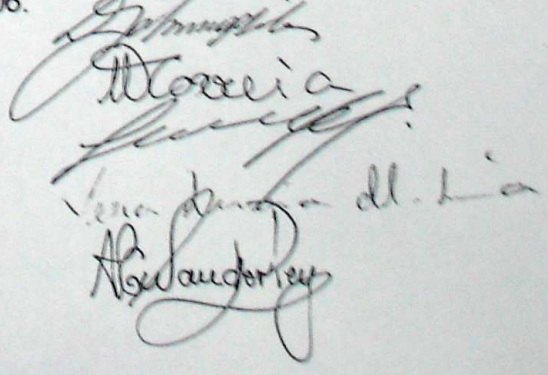
Dissertação apresentada para o cumprimento parcial das exigências para a obtenção do título de Mestre em Bioquímica pela Universidade Federal de Pernambuco.

Orientadora: Profa. Dra. Vera Lúcia de Menezes Lima
Co-orientadora: Profa. Dra. Maria Bernadete Maia

Recife, 2006

Ata da defesa de dissertação do Mestrando **Ana Catarina Rezende Leite**, realizada em 15 de fevereiro de 2006, como requisito final para obtenção do título de Mestre em Bioquímica

Às 09:10 horas, do dia 15 de fevereiro de 2006, foi aberto, no Auditório Prof. Marcionilo Barros Lins, Depto. de Bioquímica/CCB/UFPE, o ato de defesa de dissertação do mestrando **Ana Catarina Rezende Leite**, aluna do Curso de Mestrado em Bioquímica/CCB/UFPE. Iniciando os trabalhos a Profa. Dra. Vera Lúcia de Menezes Lima, Coordenadora do Curso supra citado, fez a apresentação da aluna, de sua orientadora, ela própria, de sua Co-orientadora, Profa. Dra. Maria Bernadete de Souza Maia, e da Banca Examinadora composta pelos professores doutores: Ela própria, na qualidade de Presidente, Luana Cassandra Breitenbach Barroso Coelho, Maria Tereza dos Santos Correia, ambas do Depto. de Bioquímica/CCB/UFPE e Almir Gonçalves Wanderley, do Depto. de Fisiologia e Farmacologia/CCB/UFPE. Após as apresentações, a Profa. Dra. Vera Lúcia de Menezes Lima, com a palavra convidou a aluna para a apresentação de sua dissertação intitulada: **"Avaliação da Atividade Anti-Hiperglicemiante de Extrato de *Parkinsonia aculeata* em Ratos Diabéticos"**, e informou que de acordo com o Regimento Interno do Curso, a candidata dispõe de até 50 (cinqüenta) minutos para apresentação do trabalho e o tempo de argüição para cada examinador, juntamente com o tempo gasto pelo aluno para responder às perguntas será de 30 (trinta) minutos. A aluna procedeu a explanação e comentários acerca do tema em 30 (trinta) minutos. Após a apresentação da mestranda, a Sra. Presidente convidou a Banca Examinadora para ocupar seus lugares e passou a palavra ao primeiro examinador, Prof. Dr. Almir Gonçalves Wanderley, que agradeceu o convite, fez alguns comentários e deu algumas sugestões, iniciando sua argüição. Ao final, o referido professor deu-se por satisfeito. Em seguida, a Sra. Presidente passou a palavra para a Profa. Dra. Maria Tereza dos Santos, que agradecendo o convite, fez alguns comentários e sugestões, e iniciou sua argüição. Finda a mesma, a referida professora deu-se por satisfeita. Em seguida, a palavra foi passada para a Profa. Dra. Luana Cassandra Breitenbach Barroso Coelho, que após agradecer e fazer alguns comentários, iniciou sua argüição. Ao final, a referida professora deu-se por satisfeita. Daí, a Sra. Presidente usou da palavra para tecer alguns comentários, agradecer à Banca Examinadora e parabenizar a candidata. Finalmente, a sessão foi suspensae a Sra. Presidente pediu aos presentes que se retirassem do local para que a Banca Examinadora procedesse o julgamento. Após alguns comentários, a Banca decidiu, por unanimidade, conceder a menção "**Aprovada com Distinção**". Nada mais havendo a tratar, lavrei a presente ata que vai assinada por mim, Secretário, e demais membros presentes. Recife, 15 de fevereiro de 2006.



Aos meus pais,
Flávio e Sônia pelo apoio
incondicional.

Às minhas irmãs,
Daniele e Maristela pelo constante
incentivo.

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RESUMO

Parkinsonia aculeta tem sido relatada na medicina popular como uma planta que é possuidora de ação no tratamento do Diabetes mellitus. A proposta desse estudo foi investigar a atividade dos extratos (hidroalcoólico e da fração aquosa) da *P. aculeata* em animais portadores de diabetes induzida por aloxana (150 mg/Kg) e em normoglicêmicos (NaCl 0,9%). Os extratos foram administrados nas concentrações de 250 mg/Kg e 125 mg/Kg e o veículo (água destilada, 5 mL/Kg) por via oral e diariamente durante 16 dias. Foram avaliados os níveis séricos de glicose, colesterol, triglicerídeos e colesterol-HDL, bem como os níveis urinários de glicose e uréia em 24h. Também foram avaliados o peso corporal, os níveis de comida e água ingestas, bem como o volume urinário em 24h. O peso do fígado, dos rins, do tecido adiposo epididimal e dos músculos esqueléticos: *Soleus* e *Extensor Digitorius Longus* (*EDL*) foram avaliadas, e, também, o conteúdo de glicogênio hepático. Com a finalidade de validação do modelo experimental foi realizado um grupo diabético tratado com insulina, que demonstrou, como esperado, significantes resultados na maior parte dos parâmetros metabólicos avaliados. No extrato hidroalcoólico de *P. aculeata*, onde os animais foram tratados com 125 mg/Kg, os resultados, nos animais diabéticos, demonstraram uma redução significante nos níveis de glicose sérica e urinária. O melhoramento no conteúdo do glicogênio hepático e uma redução no peso rins também foi encontrado. Nos animais normoglicêmicos tratados com o extrato, foi encontrado apenas um aumento significante no peso do *EDL*. Na fração aquosa de *P. aculeata*, os animais diabéticos tratados com 250 mg/Kg e 125 mg/Kg de extrato demonstraram uma redução significante da glicose sérica e urinária, de uréia urinaria, bem como nos níveis séricos de colesterol total, colesterol-HDL e triglicerídeos. Um melhoramento no conteúdo de glicogênio hepático e uma conservação no peso dos músculos esqueléticos: *Soleus e EDL*, bem como o significante decaimento na quantidade de líquido e comida ingestas; nos rins foi observada uma significante inibição da hipertrofia. Entretanto apenas nos animais diabéticos tratados com 125 mg/Kg da fração aquosa, foi encontrada uma redução significante no volume urinário, uma conservação no tecido adiposo epididimal, um resultado positivo no peso do fígado e no peso corporal. Os resultados significantes obtidos nos parâmetros avaliados sugerem que extrato hidroalcoólico e fração aquosa de *P. aculeata* têm ação hipoglicemiante positiva influenciando o metabolismo dos carboidratos.

ABSTRACT

Parkinsonia aculeata has been mentioned in the traditional medicine as a plant of value for the treatment of Diabetes mellitus. The purpose of this study was to investigated the activity of hydroalcoholic extract and aqueous fraction of *P. aculeata* extracts in animals with diabetic induced by alloxan (150 mg/Kg) and in normoglycemic animals (NaCl 0.9%). The extracts were administered at 250 mg/Kg or 125 mg/Kg doses or the vehicles (distilled water, 5 mL/Kg) daily, orally, for 16 days. The serum levels of glucose, cholesterol, triglycerides and HDL-cholesterol were evaluated, as well as the urinary levels of glucose and urea for 24h. The body weight, the levels of food and water intake, and the urinary volume in 24 hours also were evaluated. The weight of the liver, kidneys, epididymal adipose tissue and skeletal muscles *Soleus* and *Extensor Digitorius Longus* (*EDL*), as well the hepatic glycogen were evaluated. To validate the experimental design, a diabetic group which was treated with insulin, led to expected improvements in several abnormal parameter values. In hydroalcoholic extract of *P. aculeata* the animals were treated with 125 mg/Kg, the results, in the diabetic-treated group, demonstrated a significant reduction in the levels of serum and urinary glucose. The improvement in hepatic glycogen and a reduction in the weight of kidneys also were found. In normoglycemic group treated with the hydroalcoholic extract it was seen only a significant increase of the weight of *EDL* muscle. The aqueous fraction of *P. aculeata*, the diabetic animals, treated with 250 mg/Kg and 125 mg/Kg of extract demonstrated a significant reduction in serum and urinary glucose, and urinary urea, as well as the serum levels of total-cholesterol, HDL-cholesterol and triglycerides. An improvement in hepatic glycogen and conservation in the weight of the skeletal muscles: *Soleus and EDL*, as well a significant decrease in liquid and found intake; in the kidneys was seen a significant inhibition in the hypertrophy. However, only in the diabetic animals treated with 125 mg/Kg of aqueous extract it was found a significant reduction in urinary volume, an improvement in epididymal adipose tissue and positive results on liver weight and body weight. The significant results found in the parameters evaluated suggest that the extract hidroalcoholic and aqueous fraction of *P. aculeata* has a positive hypoglycemic action influencing at the carbohydrate metabolism.

1. INTRODUÇÃO

1.1. **Diabetes – Visão Geral**

Diabetes mellitus é uma doença metabólica caracterizada por hiperglicemia resultante de defeitos na secreção da insulina, ação da insulina, ou ambos "(HUANG, PENG, KOTA *et al*., 2005)".

Atenção considerável tem sido mundialmente dirigida ao diabetes mellitus devido a sua importância na saúde humana "(JOUAD, EDDOUCKS, LACALLI-DUBOIS *et al*., 2000)"; essa doença crônica afeta não apenas o metabolismo dos carboidratos, mas também o metabolismo de lipídeos e proteínas, "(VELTRICHELVAN e JEGDEESAN, 2002)". O diabetes afeta cerca de 29 milhões de pessoas nos EUA "(COWIE, 2003)". Indivíduos acometidos de diabetes têm uma larga redução na expectativa e na qualidade de vida "(NARAYAN, 2003)". Para todos os indivíduos nascidos a partir do ano de 2000, essa doença terá desenvolvimento em 36%.

Historicamente, o estudo da patogenia do diabetes foi delimitada no molde da endocrinologia: uma grande disfunção das células β associada com a fase de desenvolvimento do diabetes tipo I ou do tipo II, causando dessa forma uma diminuição da resposta secretória da insulina à glicose "(CERASI, 1971, SRIKANTA, 1983, TOMINAGA, 1986, DECKER, 1992)".

O diabetes tipo I é um distúrbio catabólico em que a insulina está ausente, há tendência a cetoacidose, o glucagon está elevado e as células β pancreáticas falham em responder a estímulos insulinogênicos. Resulta primariamente da destruição das células β pancreáticas em decorrência de agressão tóxica ou infecciosa às células β, em indivíduos cujo sistema imune é geneticamente predisposto a desenvolver resposta auto-imune contra células beta alteradas. Na fase que antecede a diminuição da secreção de insulina podem ser detectados anticorpos antiilhota (ICA), antiinsulina (AAI) e antianticorpo para descarboxilase do ácido glutâmico (GAD).

O Diabetes tipo II ocorre predominantemente em adultos, em sua maioria obesos, onde a insulina endógena circulante é suficiente para prevenir a cetoacidose, mas insuficiente ou relativamente inadequada pra responder aos estímulos, devido à insensibilidade dos tecidos. Soma-se a insensibilidade dos tecidos periféricos, a diminuição da secreção de insulina pelas células β frente a um estímulo de aumento da glicemia.

Outros tipos de diabetes são reportados, como demonstrado na Tabela 1; as mulheres cuja intolerância à glicose é reconhecida durante a gestação são classificadas como portadoras de diabetes gestacional "(RAMALHO, 2002)".

Tabela 1. Classificação dos tipos de diabetes.

CLASSIFICAÇÃO DO DIABETES MELLITUS	
TIPOS	**CAUSAS**
TIPO I	Destruição das células β, geralmente ocasionada por deficiência absoluta de insulina;
TIPO II	Varia de uma predominância de resistência insulínica com relativa deficiência de insulina, a um defeito predominantemente secretório, com ou sem resistência insulínica;
OUTROS TIPOS ESPECÍFÍCOS	- Defeito genéticos funcionais da célula β; - Defeito genéticos na ação da insulina; - Doença do pâncreas exócrino; - Endocrinopatias; - Induzidas por fármacos e agentes químicos; - Infecções; - Formas incomuns de diabetes imunomediado; - Outras síndromes genéticas geralmente associados ao diabetes;
DIABETES GESTACIONAL	- Fatores metabólicos e hormonais complexos envolvidos, inclusive na resistência insulínica;

Dados obtidos de "(MIGLIORINI e KETTELHUT, 1999, RAMALHO, 2002)".

Muitas propostas de estudos clínicos têm mostrado uma forte relação entre glicemia e complicações microvasculares nos dois tipos de diabetes, tipo I e tipo II "(UKPDS, 1998)", como por exemplo: na retina, glomérulo renal e nervo periférico "(BROEWNLEE, 2003)". Diabetes é também associada com a aceleração da aterosclerose afetando artérias que supri o coração, cérebro e as extremidades inferiores. Como resultado, os pacientes acometidos do diabetes tem um maior risco de infarto de miocárdio, derrame cerebral e amputação de membros "(BROWNLEE, 2001)". Hiperglicemia e resistência à insulina são fatores importantes no papel da patogênese de complicações macrovasculares "(GINSBERG, 2000)". As diferentes associações de lipídios e de distúrbios nas lipoproteínas com complicações microvasculares e macrovasculares no diabetes, também, têm sido alvo de estudo "(CHATURVEDI, FULLER e TASKINEN, 2001, SADER, NIAN e LIU, 2003)".

Os níveis de lipídeos séricos são usualmente elevados no diabetes mellitus, o que leva a um aumento representativo no risco de desenvolvimento de doenças coronarianas "(PRINCE, MENON e GUNASEKARAN, 1999)". Hormônios como o glucagon, catecolaminas e outros aumentam a lipólise "(PARI e SARAVANAN, 2002)", de modo que a acentuação da hiperlipidemia que caracteriza o estado diabético pode, portanto, ser considerada como uma conseqüência da ausência da ação inibitória de hormônios lipolíticos levando ao desenvolvimento das placas gordurosas "(AL-SHAMAONY, AL-KHARZRAJI e TWAIJI, 1994)".

Sobre circunstâncias normais, a insulina ativa a enzima lipase lipoprotéica (LPL) que tem como função hidrolisar os triglicerídeos "(TASKINEN, 1987)" transportados por lipoproteínas, como a lipoproteína de muito baixa densidade (VLDL) e Quilomícrons (QM). A LPL é normalmente acoplada ao endotélio vascular "(PHILIPS, OWENS, COLLINS *et al.*, 2005)". Portanto, com a deficiência de insulina, no quadro de diabetes, a ativação dessa enzima é afetada, levando a um quadro de hipertrigliceridemia "(PAPPAN, PAN, KWON *et al*., 2005)".

Tem sido estimado que mais de 70% dos pacientes com infarto agudo do miocárdio tem diabetes ou tolerância a glicose "(NORHAMMAR, 2002)". A patogênese da aterosclerose em indivíduos não-diabéticos tem início com disfunção endotelial, extensivamente descrito em revisões recentes "(LUSIS, 2000, LIND, 2003)". Nas artérias de diabéticos, a disfunção endotelial parece envolver tanto a resistência à insulina, quanto o desenvolvimento do quadro de hiperglicemia "(HUESH e LAW, 1998, JIANG, 1999)".

Como uma conseqüência da patologia microvascular, diabetes é umas das causas que resulta em cegueira, e em doença renal no estágio final, e diversas neuropatias. Os diabéticos são um grupo de pacientes em rápido crescimento de necessidade de diálise renal e de transplantes de rim. Mais de 60% dos pacientes diabéticos sofrem de neuropatias, onde as neuropatias periféricas contabilizam mais de 50% de todas as amputações de causas não traumáticas nos EUA "(BROWNLEE, 2003)".

1.2. Insulina

A seqüência de aminoácidos da insulina foi descoberta em 1960 por "(SANGER, 1960)", embora a síntese completa só ocorreu em 1963 por "(MEIENHOFER, SCHANABEL, BREMER *et al*., 1963)"; a estrutura tridimensional só foi demonstrada em 1972 por "(HODGKIN, 1972)".

A insulina é uma pequena proteína (M_r 5.700), com duas cadeias peptídicas retas, A e B (Figura 1) ligadas por duas pontes dissulfeto. A cadeia A possui 21 aminoácidos e a cadeia B contém 30 aminoácidos. A porção terminal carboxílica da cadeia B e as extremidades amínica e carboxílica da cadeia A formam a porção da molécula que interage com o receptor. As cadeias da insulina quando isoladas são biologicamente inativas "(RAMALHO, 2002)".

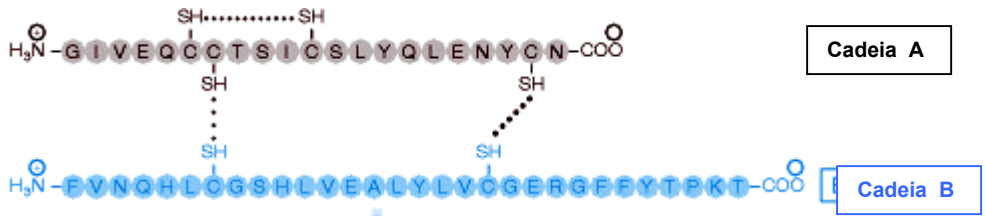

Figura 1. Estrutura da Insulina
"(STRACHAN and READ, 1999)".

A insulina é produzida no retículo endoplasmático rugoso das células β do pâncreas como precursor inativo (pré-pró-insulina) o qual é clivado a pró-insulina (86 aminoácidos) e transferido (por vesículas) para o complexo de Golgi. Nesse local essa pró-insulina é estocada juntamente com endopeptidases, que são as enzimas responsáveis por sua conversão em insulina.

A pró-insulina é convertida à insulina pela clivagem do polipeptídio conectante (peptídeo C) do terminal amínico da cadeia A com o terminal carboxílico da cadeia B. O peptídeo C contém 35 aminoácidos e não tem função biológica "(MIGLIORINI e KETTELHUT, 1999, LEHNINGER, NELSON e COX, 2002)".

A secreção de insulina é regulada por uma série de fatores; entre eles, nutrientes, hormônios gastrointestinais e pancreáticos, neurotransmissores autonômicos e glicose (Tabela 2). A glicose é o principal estímulo da secreção de insulina, sendo esse carboidrato por via oral mais eficaz para provocar secreção de insulina do que quando administrado por via parenteral. A glicose aumenta a expressão de muitos genes de célula β necessários para a síntese de proteínas, como por exemplo, o receptor que reconhece o sinal que liga a pré-pró-insulina ao retículo endoplasmático rugoso. Existem muitas nuances na liberação de insulina estimulada por glicose, onde as células β respondem de forma individual em sua sensibilidade à glicose, e somente algumas respondem em um determinado momento "(BERNE, LEVY, KOEPPEN *et al*., 2004)".

Tabela 2. Controle da secreção de insulina.

Fatores Estimulantes	**Fatores Inibidores**
- Glicose	- Adrenalina
- Frutose	- Diazóxido
- Manose	- Somatostatina
- Aminoácidos (arginina, leucina)	- Galamina
-Enteroglucagon (gastrina, secretona, pancreozimina)	

Dados obtidos de "(BERNE, LEVY, KOEPPEN *et al*., 2004)".

Os mais importantes tecidos-alvo de ação da insulina são: fígado, músculo e tecido adiposo, mas a insulina exerce efeitos reguladores em outras células. A nível hepático, a insulina atua como supressora de enzimas gliconeogênicas, inibe não só a glicogenólise, como também a lípase hormônio sensitiva que degrada triglicerídeos armazenados em ácidos graxos e glicerol, no músculo e no tecido adiposo, a insulina parece ativar a glicogênio sintetase e hexoquinase; na célula adiposa, inibe lipólise, promove a captação de glicose, que vai favorecer α-glicerol-fosfato necessário à esterificação dos ácidos graxos livres e à formação de

triglicerídeo. Ainda estimula no músculo a captação de aminoácidos e a síntese protéica, e inibe a degradação de proteína. "(CAHILL, 1971)".

Após o conhecimento da disponibilidade da insulina tornou-se evidente que o surgimento do diabetes mellitus não depende apenas da insulina, por ser esta patologia de etiologia multifatorial. A insulina foi e ainda é o principal medicamento hipoglicêmico usado no tratamento de diabetes mellitus "(JOUAD, EDDOUCKS, LACALLI-DUBOIS *et al*., 2000)". O tratamento de diabetes mellitus tipo I é baseado na insulina e/ou em agentes hipoglicemiantes orais "(VELTRICHELVAN e JEGDEESAN, 2002)". Os agentes hipoglicemiantes orais frequentemente usados na clínica prática têm várias restrições "(PROUT, 1974, HOLMAN e TURNER, 1991; WILLIAMS e PICKUP, 1991)". Hipoglicemiantes orais sintéticos podem produzir uma série de efeitos incluindo efeitos hematológicos, reações gastro-intestinais, coma hipoglicêmico e distúrbios no fígado e pulmões "(PARI e SARAVANAN, 2002)", bem como, não são convenientes para o uso durante a gravidez "(LARNER, 1985)". Plantas como medicamentos são freqüentemente consideradas como menos tóxicos e mais livres do lado do efeito colateral que os medicamentos sintéticos "(PARI e UMAMAHESWARI, 2000)".

1.3. Mecanismo Molecular da Insulina

A insulina regula tanto o metabolismo quanto a expressão gênica. O receptor da insulina na membrana plasmática é da insulina é uma glicoproteína composta de duas cadeias β unidas por duas pontes dissulfeto para formar β-α-α-β heterotetrâmero (Figura 2). A subunidade α é extracelular e a β é transmembrana com sua subunidade carboxiterminal projetando-se dentro do citosol. As cadeias α contem o domínio de ligação da insulina e os domínios intracelulares das cadeias β contem a atividade da proteína quinase "(MIGLIORINI e KETTELHUT, 1999)". Após a ligação da insulina aos receptores, estes são agregados e internalizados. A subunidade β é estimulada, levando a uma autofosforilação do receptor em vários resíduos de tirosina e na ativação da quinase. Essa tirosina quinase ativada leva a uma cascata de fosforilação, desfosforilação, que culmina com a fosforilação de fatores de transcrição nuclear, ativando-os "(LEHNINGER, NELSON e COX, 2002)", e dessa forma estimular a transcrição do gene para proteínas, que podem ser transportadores. Um exemplo desses transportadores ocorre no tecido adiposo e no músculo, onde a insulina recruta o transportador de glicose GLUT-4, que é

específico nesses tecidos, levando a um aumento de glicose de até 20 vezes pela ativação desse sistema de transporte "(BERNE, LEVY, KOEPPEN *et al*., 2004)".

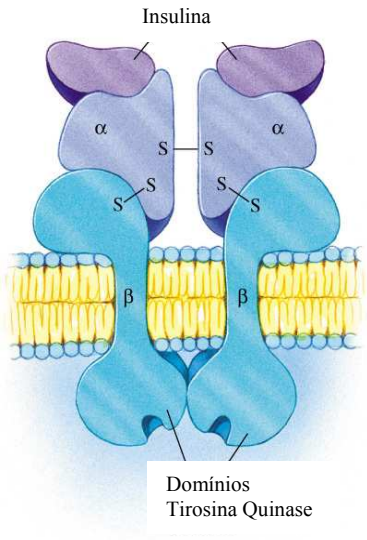

Figura 2. Estrutura Molecular do receptor da Insulina
"(HORTON, MORAN, OCHS *et al*., 2002)".

1.4. Modelo Animal

O emprego de drogas citotóxicas que causam destruição das células β das ilhotas de Langerhans do pâncreas tem sido largamente utilizado como um procedimento interessante para a obtenção de modelos de Diabetes experimental com o objetivo de estudo das alterações metabólicas decorrentes da deficiência de insulina "(SZKUDELSKI, 2001, PEPATO, FOLGADO, KETTELHUT *et al*., 2001, PEPATO, KELLER, BAVIERA *et al.*, 2002, PEPATO, BAVIERA, VENDRAMINI *et al.*, 2003, PEPATO, MORI, BAVIERA *et al.*, 2005)".

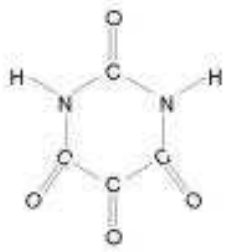

Figura 3. Estrutura química da Aloxana
"(KEFFER and RUCH, 2002)".

Dentre as drogas citotóxicas destacam-se principalmente duas: a aloxana, que é uma droga de ação seletiva em células β, sua toxicidade é devido à geração de radicais hidroxilas altamente reativas "(HALLWELL e GUTTERIDGE, 1985)", o que resulta na perda de função mitocondrial e necrose celular, e conseqüentemente, na deficiência de insulina "(DUNN e MCLETCHIE, 1943)"; segundo estudos mais recentes a aloxana ainda tem como moléculas alvo, a enzima glicoquinase e o transportador de glicose o GLUT2 "(WALDE, DOHLE, SCHOTT-OHLY *et al*., 2002)". A outra droga citotóxica de grande uso para indução do diabetes, é a estreptozotocina, menos tóxica que a aloxana, embora também leve a formação de espécies reativas de oxigênio, com conseqüente necrose celular, ela entra na célula através do GLUT2 e causa a alquilação do DNA, o que leva a uma redução na expressão do GLUT2 "(THULESEN, ORSKOV, HOLST *et al*., 1997)".

1.5. Plantas Medicinais Antidiabéticas

O uso de plantas para o tratamento do diabetes mellitus é datado em papiros dos Hebreus, anterior a 1550 a.C. Muitas plantas são usadas no tratamento do diabetes mellitus em todo o mundo "(PUSHPARAJ, TAN e TAN, 2000)". A ação hipoglicemiante plantas medicinais tem sido pouco estudada "(ALARCON-AGUILARA, ROMAN e FLORES, 1993, MAROO, VASU, AALINKEEL *et al.*, 2002)".

Depois da introdução da terapia com insulina o uso de tratamentos tradicionais para o diabetes mellitus teve um grande declínio nas sociedades ocidentais, contudo diversas plantas usadas na medicina tradicional continuam como propostas profiláticas e em conjunto com a terapia convencional "(SWANSTON FLATT, DAY, BAILEY *et al.,* 1990)". Algumas dessas plantas de tratamento tradiconal têm sido investigadas cientificamente, e a Organização Mundial de Saúde tem recomendado que o uso da fitomedicina tem demonstrado uma ação tão eficaz quanto as drogas convencionais "(WHO, 1980)".

Existem plantas que exibem propriedades similares às drogas bem conhecidas, como por exemplo, glibenclamida, um derivado das sulfoniluréias, com efeito hipoglicemiante em animais normais por estimular a liberação de insulina das células β pancreáticas, juntamente com a redução do "*clearence*" hepático da insulina "(IVORRA, PAYA e VILLAR*,* 1988; DAVIS e GRANNER, 1996)". Outras plantas agem como as biguanidas, especificamente, metmorfina "(HERMANN, SCHERSTEN, BITZEN *et al.,* 1994, STUMVOLL, NURJAN, PERRIELLO *et al.,* 1995)", que é anti-hiperglicêmica, contudo, não afeta a hipoglicemia no

estado normal "(BAILEY, DAY e TURNER, 1985)". As plantas que agem como a metmorfina "(ZHANG e TAN, 2000a, ZHANG e TAN, 2000b)" aumentam a ação da insulina por ampliar o número de transportadores de glicose, inibindo a gliconeogênese e reduzindo a absorção do intestino, e aumentando o metabolismo da glicose no fígado "(WILCOCK e BAILEY, 1990, BAILEY, 1992)".

No Brasil uma variedade muito grande de plantas têm sido utilizadas na medicina popular para o tratamento do diabetes mellitus, dentre estas podemos citar a *Bauhinia forficata*, *Eugenia jambolana*, e a *Myrcia uniflora*, bem como a *P. aculeata* L.

1.6. *P. acuelata* - Generalidades

A *P. aculeata L.*, planta da Família *Cesalpinaceae*, é uma árvore de médio porte, encontrada na região do Xingó.

Há poucos relatos na literatura sobre as ações biológicas dessa planta. Em 1947, "(SPENCER, KONIUSZY, ROGERES *et al*., 1947)", descobriram uma atividade antimalárica em um extrato hidroalcoólico de *P. aculeata.* Posteriormente, "(BHAKUNI, BITTNER, MARTICORENA *et al*., 1973)" encontraram atividade antimicrobiana de um extrato hidroalcoólico de *P. aculeata* contra *Staphylococcus aureus*, e *Sarcina lútea*.

A *P. aculeata* possui componentes químicos dentre os quais vários já foram isolados, como por exemplo: Triterpenoídes, Esteróides, Aminoácidos e Flavonóides "(AGRA, 1994)". Dentre os Flavonóides encontrados merece especial destaque os C-glicosilflavonóides "(BESSON, CHOPIN, GUNASEGARANT *et al.,* 1980, EL SAYED, AHMED, ISHAK *et al*., 1991)", que são conhecidos como estruturas responsáveis por ação hipoglicemiante e antihiperlipidêmica "(ZARZUELO, JIMINEZ, GOMES *et al*., 1996, SEZIK, ASLAN, YESILADA *et al*., 2005)".

Existem relatos de que a parte aérea (folhas, caule, flores e frutos) da *P. aculeta* são freqüentemente usadas na medicina popular da região de Xingó do Brasil como agente hipoglicemiante. Contudo, até o presente momento não foram encontrados registros na literatura sobre avaliação científica da propriedade hipoglicemiante dessa planta.

2. OBJETIVOS

2.1. Geral

Estudar o efeito dos extratos hidroalcoólico e da fração aquosa da *Parkinsonia aculeata* sobre o metabolismo de animais acometidos de Diabetes tipo I.

2.2. Específicos

1. Preparar os extratos hidroalcoólico e a fração aquosa de *Parkinsonia aculeata*;
2. Desenvolver Diabetes tipo I em modelo animal (ratos);
3. Avaliar o efeito do tratamento com os extratos de *Parkinsonia aculeata*; sobre os níveis séricos de glicose, colesterol-total, triglicerídeos e colesterol-HDL, bem como a concentração de uréia e glicose urinária e os níveis de glicogênio do tecido hepático;
4. Avaliar o tecido adiposo, tecido hepático e músculos esqueléticos de animais tratados com extratos de *P. aculeata*;
5. Avaliar a inibição da hipertrofia renal em animais tratados com extratos de *P. aculeata*;

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4. RESULTADOS

4.1. Artigo a ser submetido no periódico: Journal of Ethnopharmacology

Antidiabetic activity of hidroalcoholic extract of *Parkinsonia aculeata* in alloxan diabetic rats.

Ana Catarina Rezende Leite [a], Tiago Gomes Araújo [a], Bruno Melo Carvalho [a], Maria Bernadete Souza Maia [b], Vera Lúcia de Menezes Lima [a*]

[a] *Departamento de Bioquímica,* [b] *Departamento de Fisiologia e Farmacologia - Centro de Ciências Biológicas, Universidade Federal de Pernambuco, Recife – PE, Brazil.*

[*]Correspondence to Dr. Vera Lúcia de Menezes Lima

Departamento de Bioquímica, CCB, UFPE.

Av. Prof. Moraes Rego, S/N, Cidade Universitária, 50.670-420, Recife, PE, Brazil.

Email: vlml@ufpe.br

Phone Number: + 55- 81 – 2126 8540

Fax number: + 55 – 81 – 2126 8541

Abstract

The effect of *P. aculeata* hydroalcoholic extract (125 mg/kg) administrated for 16 days to normal and alloxan-diabetic rats was investigated. The physico-metabolic parameters measured were: body weight, food and liquid intake, urinary volume, hepatic glycogen, serum glucose, urinary glucose and urea, and the weight of epididymal adipose tissue, *soleus* and *extensor digitorum longus (EDL)* muscles, liver and kidneys. The experimental model adopted was shown to be adequate by running, a parallel treatment with insulin, which as expected led to improvement in several abnormal parameter values. The group of diabetic rats which were treated with the plant extract showed a significant reduction in serum glucose, urinary glucose. Also an improvement of hepatic glycogen and reduction of the weight of the kidneys was found in the same group. All the other parameters evaluated don't showed significant results. In the group of normoglycemic rats, the treatment with the hydroalcoholic extract induced only a significant significant increase of the *EDL* weight. The fact that normal and diabetic animals treated with *P. aculeata* hydroalcoholic extract showed significantly

changes in some of the measured parameters suggests a reduction on the glycogenolysis process and/or an increase in glycogenesis.

Key words: Antidiabetic activity; alloxan-diabetic rats; *Parkinsonia aculeata;*

1. Introduction

There is a growing awareness of role and practice of integrated medicine in field of metabolic disorders. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from a defect in insulin secretion, insulin action or both (Brownlee, 2001). Despite the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem in the world population (Brownlee, 2003). The oral hypoglycemic agents currently used in clinical practice have serious side effects (Clark et al., 2004; Rajagopalan et al., 2005). However, plant drugs are frequently considered to be less toxic and more free from side effects than synthetic ones (Pari and Umamaheswari, 2000; Calixto, 2000). Many countries use medicinal plants to control Diabetes mellitus in folk medicine (Teixeira et al., 2000; Mansour et al., 2002; Shidar et al, 2005). Experimental diabetes has the advantage that it allows the analysis of biochemical events during the induction of diabetes state and also after it has become established (Silva et al., 2002; Ravi et al., 2005). Alloxan is a diabetogenic agent which

apparently acts through formation of superoxide radicals formed by redox cycling (Szkudelski, 2001) causing irreversible damage of insulin-producing β cells (Walde et al., 2002).

P. aculeata L. (Cesalpinaceae) is a medium (Agra, 1994) tree found in the Northwest region, Xingó - Brazil, and it is used in the popular medicine as an antidiabetic agent. This study was undertaken to investigate the effects of oral treatment with hydroalcoholic extract of *P. aculeata* on some metabolic parameters of alloxan-diabetic rats and normoglycemic rats.

2. Materials and Methods

2.1. Preparation of Plant Extract

Aerial parts of *P. aculeata* were collected on Northeast region, Piranhas city, Sergipe state, Brazil. The plant was identified and a voucher specimen was deposited (number 500) in the Xingó Herbarium and was identified by Prof. H. P. Bautista (INCRA-BA). Powder of dried aerial parts (25 g) was extracted with ethanol:water (1:1; v/v). It was submitted to mechanism agitation during one hour at 23 ºC and after put in refrigerator for 24 h following of filtration. After filtration the material was lyophilized for pharmacological assays, fresh dilution of dried extract in vehicle (distilled water) was prepare on the day before the experiment and administered orally.

2.2. Animal and Treatment

2.2.1. Selection of Animals and Animal Care

The study was conducted in adult male Wistar rats weighing 180-250 g. Animals were acclimated to the experimental conditions in metabolic cages for about 3 weeks under standard environmental conditions (23 ± 1 ºC and 12-h light/dark cycle). Rats were allowed have free access to chow diet (Labina Purina – Brazil, CO) and water *ad labitum*. Six animals were used for each group of study.

2.2.2. Induction of Diabetes

Diabetes mellitus was induced in rats fasted for 16 h by intraperitoneal injection of alloxan monohydrate (Sigma, St. Louis, MO, USA) (Pari & Saravanan, 2002) dissolved in physiological saline at a dose of 150 mg/kg body weight. After three days, rats with fasting blood glucose levels greater than 200 mg/dl were considered diabetic and then included in this study. All experimental protocols were approved by the Ethic Committee for Animal studies of the Federal Univertsity of Pernambuco (UFPE).

2.3. Treatments

2.3.1. Diabetic Groups

Body weight, food and liquid intake (water), urine excretion, serum glucose, and urinary glucose and urea were measured 3 days prior and after alloxan injection and 16 days of drug treatment. At the beginning of the extract administration and every 8 days, the metabolic parameters were evaluated. The animals were allocated randomly into three groups.

The control group of diabetic non-treated animals (DNT) received water. A second group received 125 mg/kg of the hydroalcoholic extract (DT), a previous study in our laboratory has demonstrated that the daily administration of this concentration extract has better response in diabetic rats, in comparison with other concentration of extract. Similar to DT group, the treatment of diabetic insulin group (DI) started three days after alloxan injection, but it was administered twice a day (8 a.m. and 5p.m.) by subcutaneous injection of 3 units of NPH insulin (Humolin NU-100, Lilly, Brazil) for 16 days (Pepato et al., 2002). At the end of the treatment the epididymal fat-pad adipose tissue lying over the psoas, the *Soleus* and *Extensor digitorum longus* (EDL) muscles, the kidneys and the liver were removed and weighed.

2.3.2. Non-diabetic Group

The normal rats were randomly assigned into two different groups. The first group was the non-diabetic control group (NNT), which received water, and the second group was treated with the hydroalcoholic extract (NT) at a dose of 125 mg/kg body weight, daily.

2.4. Chemical and Statistical Analysis

Glucose and urea were measured by enzymatic methods (Srivastava et al., 2001). Hepatic glycogen was extracted with 30% KOH and precipitated with alcohol (Carrol et al., 1956) and the quantity recovered determined by the colorimetric anthrone method of Collowick and Kaplan (1957).

Data were statistically evaluated by using one-way ANOVA, followed by post hoc Dunett C test using 11.0 version of SPSS computer software and by using the Student's *t*-test at a significance level of $P<0.05$.

3. Results

The physiological variables, such as: body weight, food and liquid intake, and urinary volume of the DNT and DT, as NNT and NT groups are displayed in Table 1 and Table 2 respectively. Indeed just urinary volume, in DT, presents a significant decrease, at the end of the16 days of treatment with the hydroalcoholic extract of *P. aculeata*, the others parameters in the groups were found to have no significant difference in the metabolic parameters measured (Table 1 and 2). The values of these same metabolic parameters in DI group, before and after the treatment with insulin are shows in Table 3. As expected, the results showed that the metabolism of DI improved considerably after the insulin treatment.

In the present work the results showed that the 16 days of treatment with the hydroalcoholic extract of *P. aculeata* significantly reduced serum glucose levels (Figure 1) in DT group when compared to DNT group. In the same figure, can be also seen the effect of the treatment with the hydroalcoholic extract of *P. aculeata* on the levels of urinary glucose en DT group when compared to DNT group. The DI group in the present work showed a good response in alloxan-diabetic rats after the period of treatment in the levels of serum and urinary glucose. The results from the NT group indicated that the hydroalcoholic extract of *P. aculeata* has no effect on serum glucose and urinary glucose when the animal is normoglycemic.

A significant difference, about 4-folds, was observed between group DNT and DT for hepatic glycogen content, measured on the end of the 16 days of treatment with the hydroalcoholic extract of *P. aculeata*, while this variable NNT and NT was unchanged by the treatment.

The weight of skeletal muscles *Soleus* and *EDL,* in DT group (Table 4) was similar to that found in DNT group, and this indicate that the hydroalcoholic extract of *P. aculeata* did not lead to any improvement in protein metabolism. Nevertheless, in NT group was observed a significant increased in the weight of *EDL* (Table 4).

Examing Table 4, we also can see that there was a significant decreased in the weight of the kidneys of DT group as compared to DNT group. Normoglycemic groups did not show any significantly change in this parameter. Moreover, there was no significant effect of the treatment with *P. aculeata* hydroalcoholic extract in the weight liver and epididymal adipose tissue from the group of diabetic rats and normoglycemic rats.

4. Discussion

P. aculeata is used in folk medicine in Brazil to treat diabetes, yet there have been no attempt to determine scientifically if it has an antidiabetic effect. It is well established that the one of the constitutive in the phytochemistry of *P. aculeata* is C-glycosylflavones (Besson et al., 1980; El Sayed et al., 1991), this compound is known as to have hypoglycemic and antihyperglycemic action (Sezik *et al*., 2005; Zarzuelo *et al*., 1996).

In the experimental model adopted in this work, the animals injected with alloxan presented clear symptoms of severe diabetes, in the altered values of parameters known to suffer changes in this illness (Table 1 and 2, Figure 1). To validate the experimental model a group of diabetic animal was treated with insulin, which led to correction in most of the altered parameters (Table 3).

By reducing serum and urinary glucose in DT group (Figure 1) we could say that the hydroalcoholic extract of *P.aculeata* had a beneficial effect on carbohydrate metabolism in diabetic rats. The improved levels of glucose seen after treatment with hydroalcoholic extract of *P. aculeata* can be attributed to stimulation of glycogen synthesis and/or inhibition of

glycogenolysis, as it was found a benefice in the level of liver glycogen (Table 4). Glycogen is the primary intracellular storable form of glucose, and its levels in various tissues are a direct reflection of insulin activity because insulin promotes intra cellular glycogen deposition by stimulating glycogen synthesis and inhibiting glycogen phosphorylase. Maiti et al. (2005) found similar results when studying the effect of an aqueous extract of seeds of *Tamarindus indica* for 14 days of treatment; and they found significant reduction in serum glucose and glycogen levels in streptozotocin-induced diabetic rats.

We could say that hydroalcoholic extract of *P.aculeata* do not has a beneficial effect in protein metabolism in alloxan-induced diabetic rats, when we verified that the skeletal muscles suffered a reduction. The insulin deficiency develop the reduction on aminoacids caption and proteolitic pathway alterations, these modifications cause muscular marked proteolysis responsible for muscular mass decrease (Cahill, 1971).

The unaltered masses of epididymal adipose tissue observed in this study about fat metabolism agree with the study by Pepato et al. (2002) who treated streptozotocin-induced diabetic rats with *Bauhinia forficata* decoction, for about 1 month, and they found no significant alteration in epididymal adipose tissue, although a significant reduction on serum and urinary glucose was observe.

Diabetic animals tend to show renal hypertrophy because the entry of glucose in renal tissue is not dependent on action of insulin, in the diabetes; the hyperglycemia causes an increase in the entry of glucose. A key morphological change associated with sustained hyperglycemia was the accumulation of glycogen granules in distal tubules, which leads to the renal hypertrophy (Kang et al., 2005). In our finds in this work, the weight of the kidneys of the DT group significantly decreased, but the weight of liver was unchanged. The literature regarding the effect of diabetes on liver weight is contradictory as some workers have show

an increase in hepatic weight in animals (Sadique et al., 1987) while others have reported no change (Musabayne et al., 2005).

The results observed in NT group in our study, showing just a significant improvement in the weight of *EDL* muscle, no more significant changes in any metabolic or biochemistry parameters evaluated were found, after the 16 days of treatment with *P. aculeata* hydroalcoholic extract. We could conclude that the treatment with *P. aculeata* hydroalcoholic extract affects the metabolism of carbohydrate and proteins in alloxan-induced diabetic rats.

5. Conclusion

In summary, this investigation about administering hydroalcoholic extract of *P. aculeata* by 16 days revealed a beneficial effect on some biochemical parameters related to carbohydrate and protein metabolism; and in urinary volume. A possible mechanism might involve a reduction on glycogenolysis and/or an increase in glycogenesis, when is observed the levels of serum and urinary glucose and hepatic glycogen concentration. Nevertheless, new studies are need in order to elucidate the mechanism of the action of the *P. aculeata* hydroalcoholic extract, and also to isolate and characterize the active compounds.

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Table 1 - Metabolic parameters of diabetic rats during the oral treatment with the hydroalcoholic extract of *P. aculeata*.

Metabolic parameters	Days of treatment 0		8		16	
	DNT	DT	DNT	DT	DNT	DT
Body Weight (g)	159.2±4	162.3±8	163.3±6	164.3±8	147.8±6	156.9±10
Liquid Intake (mL/24 h)	161.6±14	129.6±8	172±18	79±21	193.8±7	140.1±8
Food Intake (g/24 h)	25.7±2	25.6±3	28.2±3	22.2±2	35.6±0	32.8±1
Urinary Volume (mL/24 h)	92.3±4	83.5±2	123.2±7	77.8±8	136±1	78±4*

DNT: diabetic non-treated; DT: diabetic-treated. The effect of *P. aculeata* on several metabolic. The levels of fluid intake, urinary volume, food intake, body weight. All values represent means ± S.E.M. (n = 6), employing one-way ANOVA.

Table 2 - Metabolic parameters of normoglycemic rats during the oral treatment with the hydroalcoholic extract of *P. aculeata*.

Metabolic parameters	Days of treatment 0		8		16	
	NNT	NT	NNT	NT	NNT	NT
Body Weight (g)	163±3	201±9	203±8	242.6±9	224.8±6	258.4±11
Liquid Intake (mL/24 h)	37.5±6	41.6±1	24.1±5	42.3±1	28±1	32.7±2
Food Intake (g/24 h)	18±3	20.8±1	20.3±3	25.3±0	18.2±1	24.5±1
Urinary Volume (mL/24 h)	5.3±1	10.5±0	7.3±0	8.3±0	6.7±1	7.5±0

NNT: normoglycemic non-treated; NT: normoglycemic treated. The effect of *P. aculeata* on several metabolic and biochemical parameters. The levels of fluid intake, urinary volume, food intake, body weight. All values represent means ± S.E.M. (n = 6), employing one-way ANOVA.

Table 3 - Metabolic parameters of diabetic rats before and after 16 days of treatment with insulin.

Metabolic parameters	Before insulin treatment	After insulin treatment
Body Weight (g)	168.3±2.6	235.5±4.5*
Fluid intake (mL/24h)	129.3±11.4	70.3±5.9*
Food intake (g/24h)	23.8±1.7	26.5±1.2
Urinary volume (mL/24h)	86.8±1.7	40±4.7*
Urinary glucose (mg/dL)	525.2±0.6	496.6±0.6*
Urinary urea (g/24h)	56.6±1.3	15.2±3.3*
Serum glucose (mg/dL)	1044.6±17.6	469.9±40.6*

All the values represent means± S.E.M. (n = 6). Measurements before insulin treatment were taken 3 days after alloxan injection. *$p<0.001$ compared with before insulin treatment using paired Student's t test.

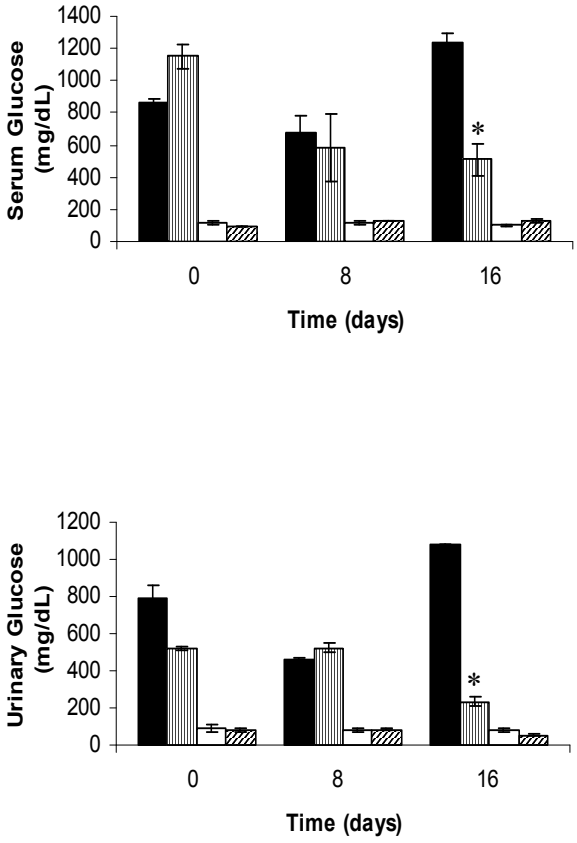

Figure 1. The effect of *P. aculeata* on the levels of serum glucose and urinary glucose. All values represent means ± S.E.M. (n = 6). (■) DNT, (◫) DT, (□) NNT and (▨) NT.

Table 4 - Weight of liver, kidney, epididymal adipose tissue, and *Soleus* and *EDL* muscles of non-diabetic and diabetic rats treated or untreated with *P. aculeata* hydroalcoholic extract.

	Weight of tissues (g) per 100g of body weight			
	Non-diabetic groups		Diabetic groups	
Tissue	NNT	NT	DNT	DT
Hepatic glycogen (mg%)	2.23±1.40	2.88±0.7	0.07±0.01	0.29±0.06**
Liver	4.71±0.21	4.80±0.16	5.94±0.20	5.37±0.20
Kidneys	0.44±0	0.40±0	0.76±0	0.49±0.03**
Epididymal	0.92±0.05	0.96±0.07	0.14±0	0.14±0.01
Soleus	0.05±0	0.05±0	0.06±0	0.06±0
Extensor digitorum longus	0.05±0	0.05±0*	0.04±0.06	0.05±0

NNT: normoglycemic non-treated; NT: normoglycemic treated; DNT: diabetic non-treated; DT: diabetic treated. Values represent means ± standard error (n = 6), using Student's t test. Intergroup differences (*$p<0.05$, **$p<0.001$).

4.2. Artigo a ser submetido no periódico: Biological and Pharmaceutical Bulletin

Biol. Pharm. Bull. - Regular Article
Medicinal Chemistry

Biochemical studies on the effects of aqueous fraction extract from *Parkinsonia aculeata* L. in alloxan-induced diabetic rats

Ana C. R. Leite, [a] Tiago G. Araújo, [a] Bruno M. Carvalho, [a] Nicácio H. Silva, [a] Vera L.M. Lima, [a] and Maria B.S. Maia [*,b]

[a] *Departamento de Bioquímica,* [b] *Departamento de Fisiologia e Farmacologia, Centro de Ciências Biológicas, Universidade Federal de Pernambuco (UFPE), Cidade Universitária, 50670-901, Recife-PE, Brazil.*

* Corresponding author: Tel: +55 81 2126 8530, Fax: +55 81 2126 8976. E-mail addresses: mbsm@ufpe.br

SUMMARY

The antidiabetic effect of *Parkinsonia aculeata* aqueous fraction (PAEF) extract was investigated in alloxan diabetic rats. The physico-metabolic parameters measured were: body weight, food and liquid intake, urinary volume, hepatic glycogen, serum glucose, total cholesterol, HDL-cholesterol, triglycerides, urinary glucose and urea, and the weight of epididymal adipose tissue, liver, kidneys and the skeletal muscle (*soleus* and *extensor digitorum longus*). Oral administration of PAEF (125 or 250 mg/kg; v.o.) for 16 days exhibited a significant reduction in serum and urinary glucose, urinary urea, total cholesterol, HDL-cholesterol and triglycerides in alloxan diabetic rats. A improvement of hepatic glycogen, a decrease of liquid and food intake, and a significantly positive actions in the weight in the skeletal muscles (*soleus* and *extensor digitorum longus*) and kidneys were also observed. But only diabetic group treated with PAE at a dose of 125 mg/kg showed significant reduction in urinary volume, body weight, an improvement of epididymal adipose tissue and a positive action in liver weight. The results of this work confirm the traditional use of *P. aculeata* and suggest its clinical use may be useful for the treatment of diabetes mellitus.

Key word: Antidiabetic plant; alloxan-induction; *Parkinsonia aculeata*; Cesalpinacea.

Despite of efficient synthetic drugs (biguanides, sulphonylureas etc) offer effective treatment options for diabetes mellitus treatment, the herbal therapy is note rare among to diabetic patients. In many developing countries, where the public health system is insufficient to provide basic pharmaceutical and medical care to the population, it may be presented as an option to or in conjunction with conventional medicine for prevent and control diabetes-related complications. Several studies carried out with numerous herbs having folk medicine reputation for antidiabetic potency (*Tithonia diversifolia*, [1)] *Cissus sicyoides*, [2)] *Eugenia jambolana*, [3)] *Enicostemma littorale*, [4)] *Occimum sanctum*, [5)] and *Bauhinia forficate*, [6)] *Tamarindus indica*, [7)] *Pterocarpus marsupium*, [8)] *Genista tenara* [9)]) have shown promising results on experimental model of diabetes. Although no standardized active principles from these plant specie have been yet identified and isolated, and most of the above mentioned findings were obtained from animal model of diabetes, these primary information are very important in view that they give a scientific basis to research towards clinical evaluation (the beneficial effects on a person's health must be the focus of clinical herbal investigation) and developing new and effective antidiabetic drugs according their different therapeutic potentials.

Parkinsonia aculeate L. (Cesalpineaceae) is a medium tree (4-6 m) found in the Xingó region (semi-arid area) in Northeast of Brazil, recognized by local population as an antidiabetic agent. [10)] According review report indicates, no previous pharmacological validation of this plant, which provides scientific understanding and research reference about its ethnomedicinal tradition has been carried out. The main objective of the present work was to determine the antidiabetic effect produced by subchronic administration (16 days) of oral administration of PAEF in alloxan-induced diabetic rats, a model system considered as an important tool to study the pathophysiological mechanisms of diabetes mellitus and hypoglycemic activity of plants. [11, 12, 13)] The effect of *P. aculeata* is compared with insulin as a reference hypoglicaemic drug.

MATERIAL AND METHODS

Plant Material: Aerial parts of *P. aculeata* were collected from Xingó (Sergipe, Brazil). Avoucher specimen (nº 500) authenticated is deposited in Xingó herbarium (Canindé do São Francisco, Sergipe, Northeast Brazil).

Preparation of the aqueous extract fraction (PAEF): For a guaranteed extraction of hydrosoluvel substances, dried and powered aerial part of *P. aculeata* (25 g) were successively extracted with ether, chloroform and acetone in a soxtlet apparatus, at 40 ºC. The next extraction was conducted with distilled water at room temperature. All extracts fraction was submitted to mechanism agitation during one hour and after put in refrigerator for 24 h following of filtration. After filtration the material was lyophilized for pharmacological assays, fresh dilution of dried extract in vehicle (distilled water) was prepare on the day before the experiment and administered orally through orogastric at the following doses of 125 or 250 mg/kg the volume of the vehicle for all the above doses was kept constant at 1ml.

Animals and experimental induction of diabetes: Adult male Wistar rats (180 to 250 g) were housed in a metabolic cage and kept under standard environmental conditions (22±3 ºC; 12/12 h light/dark cycle. They were fed with standard diet (Labina Purina – Brazil CO) and water *ad libitum*. All animals were allowed to adapt to metabolic cages for 3 days, after which they were fasted overnight and 150 mg/kg of alloxan monohydrate (Sigma, St. Louis, MO, USA) freshly dissolved in normal saline was injected intraperitoneally. After alloxan treatment, all animals were returned to their cages and given free access to food and water. Blood glucose levels were measured 3 day after alloxan injection and used as parameter to obtain matching pairs of rats with diabetes of a similar level of severity. Only rats with fasting blood glucose levels greater than 200 mg/dl were considered diabetic and then included in this study. The blood concentration of glucose in normoglycemic rats was in the range of 95 mg/dl. Diabetic rats were randomly assigned to four different groups (n = 6 animal/groups). The control group was assigned to diabetic control (DC), which received destilated water (5 ml/kg; v.o.); treated groups received orally PAEF (125 mg/kg (DT125) or 250 mg/kg (DT250). The diabetic insulin group (DTI) was treated twice a day by subcutaneous injection of 3 units of NPH insulin (Humolin NU-100, Lily, Brazil) during 16 days. All treatments started on the 3rd day after alloxan injection.

Determination of parameters: Blood samples were withdrawn from the cavernous sinus with a capillary for biochemical parameters determination. Serum glucose, cholesterol, triglycerides, HDL-cholesterol, urinary glucose and urea were measured by enzymatic methods (Labtest Diagnostica – Brazil/SA). Hepatic glycogen was extracted with 30% KOH and precipitated with alcohol [14] and the quantity recovered determined by the colorimetric anthrone method. [15] All the parameters were evaluated each 8 days, until to the 20th day (16st day of treatment). After the last measurement the rats were sacrificed. The epididymal fat pad adipose tissue lying over psoas, the *soleus* and *extensor digitorum longus* (*EDL*) muscles and the kidney were removed and weighed and the liver was removed to measure its glycogen content.

Statistical analysis: Values are expressed as means ± S.E.M. Data were statistically evaluated by using Student's *t*-test. Differences were considered significant when $p<0.05$

RESULTS

Effect of PAEF on blood glucose level. Oral administration of PAEF (125 or 250 mg/kg) for 16 days resulted in significant reduction in blood glucose. PAEF at dose of 125

mg/Kg body weight exhibited better sugar reduction (73.2%) than that verified with 250 mg/kg body weight (69%) or that produced by the standard drug, insulin (69%) at the same period (Fig 1A).

Effect of PAEF on urinary glucose level. There was a significant reduction in urinary glucose level in respect the rats of both diabetic treated groups (DT125 and DT250) when compared to DC group (Fig 1B). Once again the reduction was more pronounced in DT125, and was statistically comparable to that observed in DTI group.

Effect of PAEF on urinary urea level. Supplementation of PAEF to DT125 and DT250 group for 16 days resulted in a significant decreasing in urinary urea level. The urinary urea value exhibited by DT125 (14.72 g/24h) group was neighbor to that presented by DTI group (15.25 g/24h) (Fig 1C).

Serum lipid profile. Beneficial effects of PAEF on serum lipids, one of the major cardiovascular risk factors in type 2 diabetes mellitus, can be observed from lipid-related data registered in Table 1. Compared with the control values (DC), the DT250 group showed significant reduction in the serum levels of total cholesterol, HDL-cholesterol and triglycerides, while the DT125 group exhibited significant reduction only in serum triglycerides levels.

Glycogen level in liver. When compared with DC group, there was a significant elevation in hepatic glycogen content in both diabetic treated groups (DT125 and DT250). Here, this effect was more pronounced in the DT250 group, and was statistically comparable to that observed in DTI group (Table 1).

The effect of PAEF on physiological variables. The effect of PAEF on physiological variables (body weight, food and liquid intake and urinary volume) are displayed in Figure 2. An increase in body weight (Fig A) as well a significant reduction in urinary volume (Fig B) was found in DT125 and DTI group. The groups DC, DT125, DT250 and DIT showed a significant decreasing in food (Fig C) and liquid intake (Fig D).

Effect of PAEF on weight of liver, kidney, TAE, EDL and *soleus*. The effect of oral administration of PAEF (125 or 250 mg/kg) on weight of liver, kidney, TAE, EDL and *Soleus* is shown in table 2. The DT125 group showed significant decreasing in liver and kidney weights, and an increasing in TAE, EDL and *soleus* weights, when compared with the control (DC) value. Concerning the DT250 group, the animals showed a significant improvement in skeletal muscles *Soleus* and *EDL*. On the other hand, no difference in liver and TAE weight were observed between this group and the control (DC).

DISCUSSION

An aqueous extract fraction of *P. aculeata* (PAEF) was screened to explore the scientific basis of its utility for correction of hyperglycemia and hyperlipidemia in alloxan-induced diabetic rats. This specimen has been reported in folk medicine to be useful in diabetes, but there have been no attention to determine scientifically its antidiabetic effect. However, one of phytochemical member from *P. aculeate*, the compound C-glicosylflavones, [16, 17] has been reported as hypoglycemic and antihiperglycemic agent, [18, 19] and is expected that in this aqueous fraction had more of this compound, because the carbohydrate radical is hydrosoluble.

In all the experiments with PAEF (125 or 250 mg/g body weight) definite blood glucose lowering and serum lipid profile have been confirmed in alloxan diabetic rats

The diabetic condition which we induced in the rats by giving them alloxan was rated as severe, [20] as observed by the decrease of body weight and hepatic glycogen, as well as by the increase in the levels of serum glucose, urinary glucose and urea and serum lipids; seen

when we compared the (DIT) group to the others groups (Figure 1, Tables 1, 2 and 3). That our model was appropriate is shown by the fact that when the rats were treated with insulin there was an improvement in the great majority of the variables classically affected by diabetes, while the administration of aqueous fraction of *P. aculeata* produced significant improvement in the diabetes induced by alloxan in this rats as demonstrated in the variables related to carbohydrate, lipids and protein metabolism.

In the present study we could state that the aqueous fraction of *P.aculeata* has a positive effect in serum and urinary glucose in alloxan-diabetic treated rats (Figure 1). Similar results as ours was observed, [6] it were found significant decrease in plasmatic and urinary glucose, in diabetic rats induced by streptozotocin (40 mg/kg into the jugular vein) treated with a decoction of *B. forficata*, belongs to the family Caesalpinaceae (formally Leguminosae), for 31 days. The improvement found in our study in the content of hepatic glycogen in alloxan-diabetic treated groups (Table 2) shows that the treatment with aqueous fraction of *P. aculeata* has a beneficial effect in carbohydrate metabolism. This prevention of depletion of glycogen in the liver of the alloxan- diabetic animals may be related to a increasing in glycogenesis and/or a reduction in glycogenolysis.

The metabolism of proteins was apparently affected by the aqueous fraction of *P. aculeata*. The reduction on the levels of urinary urea in the alloxan-diabetic treated groups (Figure 1) might indicate that, this aqueous fraction exerts its effect of reducing hyperglycemia by the inhibition of gluconeogenesis. This affirmative agrees with the fact that in our work, there were significant results in *Soleus* e *EDL* skeletal muscles weight (Table 3), with the treatment in alloxan-diabetic with aqueous fraction extract of *P. aculeata*. It well known that the insulin is a key in the proteolytic alterations and on the caption of aminoacids, the decrease in insulin signaling cause muscular proteolysis, which is responsible for muscular mass decrease.

Diabetic animals has a rise in blood sugar is accompanied with the increase in total cholesterol, LDL-c, VLDL-c, triglycerides and fall of HDL-c. [11] However, the diabetic groups treated with aqueous fraction of *P. aculeata* extract exhibited hypolipidemic effect (Table 1), which results in a decrease in total cholesterol and triglycerides, and also in an increase in HDL-c serum levels. This suggests that aqueous fraction of *P. aculeata* extract can prevent or be helpful in reducing the complications of lipid profile seen in some diabetics in whom hyperglycemia and hypertriglyceridemia coexist quite frequently.

T. indica is a plant which comes from the same family (Caesalpinaceae) as ours plant in this study (*P.aculeata*). Treating streptozotocin-diabetic rats for 14 days, [7] with an aqueous extract of seeds of *T. indica*, was observed a significant reduction in serum cholesterol and triglycerides levels, and these parameters were resettled toward the normoglycemic levels. Also in HDL-c, there was a significant elevation of this lipoprotein in serum and was resettled to the normoglycemic level. These results agree with ours present findings.

By looking another parameter into fat metabolism in these alloxan-diabetic treated animals, we could observe that this plant has appreciable response, in the epididymal adipose tissue (TAE) from DT125 group (Table 2). The decrease at the serum levels of total cholesterol, triacylglycerols and the increase of HDL-c, agrees with the positive effect in TAE and it is probable that the mobilization of fat or resynthesis of triacylglycerols are involved.

In this work, we observe a reduction in the weight of the liver in the alloxan treated animals (DT 125) (Table 2); [21] found similar results in alloxan-diabetic animals treated with cotton seed aqueous extract, an increase in the weight of the liver in these animals and a enhance in the liver glycogen. Another beneficial result of the treatment in alloxan-diabetic animals with the aqueous fraction of *P. aculeata*, was show in the weight of the kidneys (Table 2), their were significantly reduced. The renal pathogenesis is related to duration of

diabetes, the most devastating complication with diabetes is nephropathy. A key morphological change associated with sustained hyperglycemia was the accumulation of glycogen granules in distal tubules, which leads to the renal hypertrophy. [22)]

In summary, it was demonstrated that the subchronic oral administration of the aqueous fraction of *P. aculeata* extract to alloxan-diabetic rats was useful for the treatment of diabetes induced by alloxan, because there were significant positive changes in the biochemical and physiological parameters related to carbohydrate, protein and lipids metabolism investigated. Other pharmacological, biochemical, histological and chemical studies in animals are needed to elucidate the mechanism of the action of *P. aculeata* extract.

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Figure 1. The effect of *P. aculeata* on the levels of serum glucose (A), urinary glucose (B) and urea (C). All values represent means ± S.E.M. (*n* = 6). (▩) NNT, (■) DC, (▥) DT250, (□) DT125 and (▨) DTI. For statistical analysis was employing unpaired Student's *t* test (*p<0.05).

Figure 2. The effect of *P. aculeata* on the levels of fluid intake (A), urinary volume (B), food intake (C) and body weight (D). All values represent means ± S.E.M. (*n* = 6). (▩) NNT, (■) DC, (▥) DT250, (□) DT125 and (▨) DTI. For statistical analysis was employing unpaired Student's *t* test (*p<0.05).

Table 1 - Biochemical variables of alloxan-diabetic groups, after 16 days of treatment with aqueous fraction extract of *P. aculeata.*
NNT: normoglycemic non-treated group. Values represent means ± S.E.M. (*n* = 6). Intergroup differences employing unpaired Student's *t* test (*p<0.05; **p<0.01; ***p<0.001).

Table 2 - Weights of liver, kidneys, epididymal adipose tissue and *Soleus* and *EDL* muscles of alloxan-diabetic groups, after 16 days of treatment with aqueous fraction extract of *P. aculeata*.
NNT: normoglycemic non-treated group. Values represent means ± S.E.M. (*n* = 6). Intergroup differences using unpaired Student's *t* test (*p<0.05; **p<0.01; ***p<0.001).

Fig. 1.

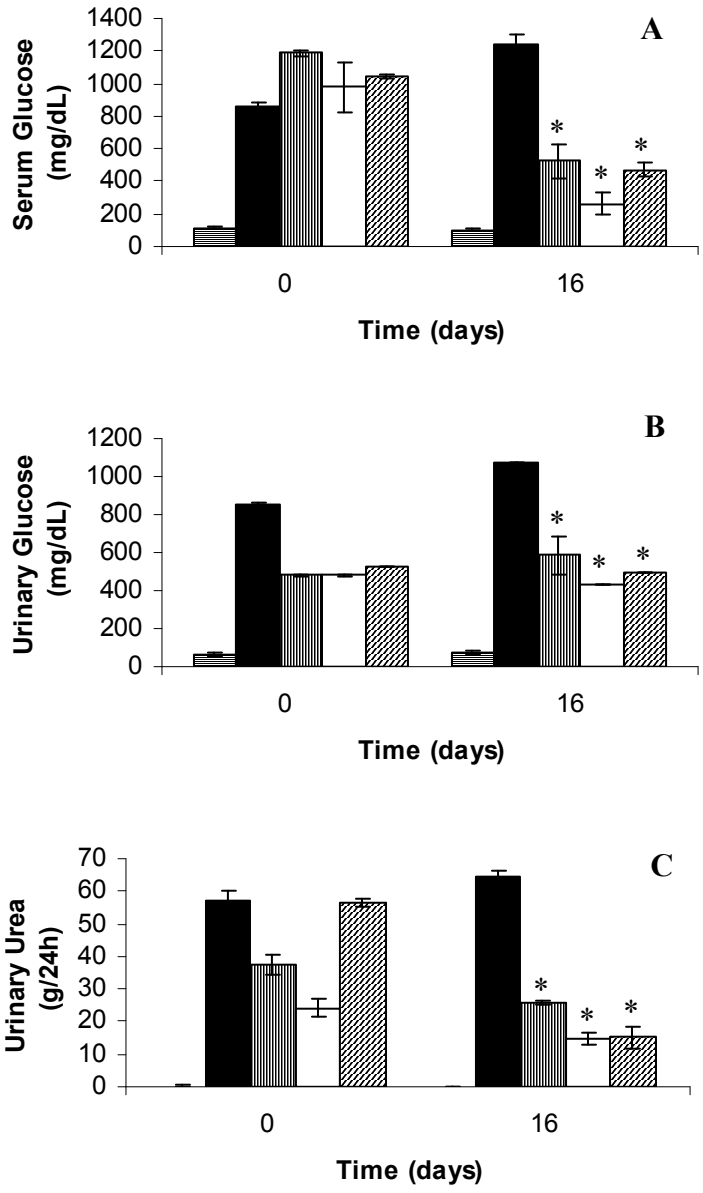
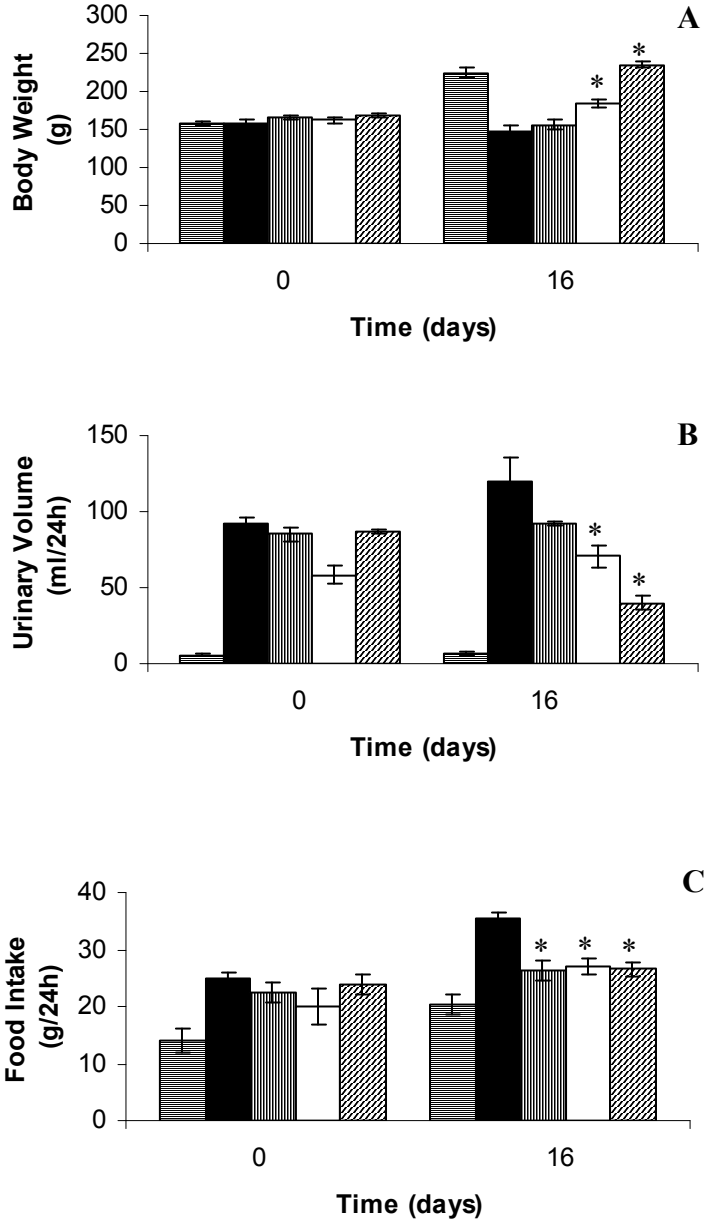

A
Serum Glucose (mg/dL)
1400
1200
1000
800
600
400
200
0
0
16
Time (days)
B
Urinary Glucose (mg/dL)
1200
1000
800
600
400
200
0
0
16
Time (days)
C
Urinary Urea (g/24h)
70
60
50
40
30
20
10
0
0
16
Time (days)

Fig. 2.


A
Body Weight (g)
300
250
200
150
100
50
0
0
16
Time (days)
B
Urinary Volume (ml/24h)
150
100
50
0
0
16
Time (days)
C
Food Intake (g/24h)
40
30
20
10
0
0
16
Time (days)

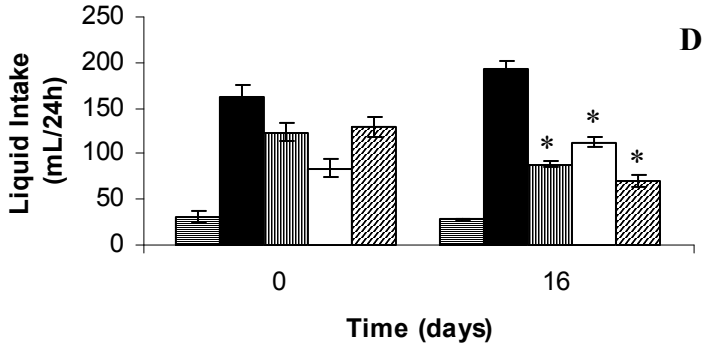


Table 1.

Metabolic parameters	NNT	DC	DT250	DT125	DTI
Hepatic glycogen (mg%)	2.23±1.40	0.05±0.01	0.29±0.04***	0.51±0.14* *	0.65±0.08***
Triglycerides (mg/dL)	90.86±5.71	241.83±15.46	88.10±12.60*	121.39±9.26*	144.15±18.47*
Cholesterol (mg/dL)	64.90±2.45	70.59±4.63	38.24±3.94*	56.28±6.45	79.71±3.43
HDL-cholesterol (mg/dL)	38.27±1.52	33.46±2.00	26.80±1.40*	30.29±1.32	42.26±1.89*

Table 2.

	Weight of tissues (g) per 100g of body weight				
Tissue	NNT	DC	DT250	DT125	DTI
Liver	4.71±0.21	5.94±0.20	5.44±0.11	5.22±0.03**	5.30±0.33
Kidneys	0.44±0	0.76±0.04	0.10±0.01***	0.49±0.03***	0.47±0.01***
TAE	0.92±0.05	0.14±0.01	0.13±0	0.45±0***	0.86±0.07***
Soleus	0.05±0	0.06±0	0.07±0*	0.08±0*	0.05±0**
EDL	0.05±0	0.04±0	0.05±0*	0.05±0**	0.05±0

5. CONCLUSÕES FINAIS

- Os resultados que extratos hidroalcoólico e a fração aquosa da *P. aculeata* demonstram ter uma ação muito positiva na maioria dos parâmetros metabólicos avaliados;
- Tendo provavelmente um mecanismo de ação que está envolvido com a redução da glicogenólise e/ou um aumento da glicogênese;
- Causando assim um melhoramento no metabolismo dos carboidratos, proteínas e lipídios, em animais diabéticos induzidos por aloxana;
- O principio ativo que provavelmente está envolvido nessa ação é o C-glicosilflanóide, que por ter um carboidrato como radical, e esse sendo hidrossolúvel, possivelmente estará em maior concentração na fração aquosa;

6. ANEXOS