**UNIVERSIDADE FEDERAL DE PERNAMBUCO**
**CENTRO DE CIÊNCIAS BIOLÓGICAS**
**MESTRADO EM BIOQUÍMICA E FISIOLOGIA**

**JOSÉ OLIVÁ APOLINÁRIO SEGUNDO**

# EFEITO DO TRATAMENTO COM NOVOS DERIVADOS IMIDAZOLIDÍNICOS E PRAZIQUANTEL SOBRE OS LIPÍDIOS DE CAMUNDONGOS INFECTADOS POR *SCHISTOSOMA MANSONI*

**RECIFE, 2008**

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Dissertação apresentada ao Programa de Pós-graduação em Bioquímica e Fisiologia como requisito final para obtenção do grau de Mestre em Bioquímica e Fisiologia pela Universidade Federal de Pernambuco.

Orientadora: Profa. Dra. Vera Lúcia de Menezes Lima

Co-orientadora: Profa. Dra. Suely Lins Galdino

**RECIFE, 2008**

**Apolinário Segundo, José Olivá**

**Efeito do tratamento com novos derivados imidazolidínicos e praziquantel sobre os lipídios de camudongos infectados por *Schistosoma mansoni* / José Olivá Apolinário Segundo. – Recife: O Autor, 2008.**

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**Defendida em 25 de Fevereiro de 2008.**

BANCA EXAMINADORA:

_____

Professora Dra. Patrícia Maria Guedes Paiva
Departamento de Bioquímica - UFPE (Membro interno)

_____

Professora Dra. Luana Cassandra Breitenbach Barroso Coelho
Departamento de Bioquímica - UFPE (Membro interno)

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Professora Dra. Mônica Camelo Pessoa de Azevedo Albuquerque
Departamento de Medicina Tropical - UFPE (Membro externo)

_____

Professora Dra. Vera Lúcia de Menezes Lima
Departamento de Bioquímica – UFPE (Presidente)

Ata da defesa de dissertação do Mestrando **José Olivá Apolinário Segundo,** realizada em 25 de fevereiro de 2008, como requisito final para obtenção do título de Mestre em Bioquímica e Fisiologia da UFPE.

Às 09:40 horas, do dia vinte e cinco de fevereiro de 2008, foi aberto no Auditório Prof. Marcionilo Lins – Depto. de Bioquímica, do Centro de Ciências Biológicas da Universidade Federal de Pernambuco o ato de defesa de dissertação do mestrando **José Olivá Apolinário Segundo**, aluno do Curso de Mestrado em Bioquímica e Fisiologia/CCB/UFPE. Iniciando os trabalhos a Profa. Dra. **Vera Lúcia de Menezes** fez a apresentação do aluno, sua orientadora, ela própria, sua co-orientadora Profa. Dra. Suely Lins Galdino, bem como da Banca Examinadora composta por ela própria, na qualidade de Presidente e as professoras doutoras: Patrícia Maria Guedes Paiva, Luana Cassandra Breitenbach Barroso Coelho, ambas do Depto. de Bioquímica/UFPE, e Mônica Camelo Pessôa de Azevedo Albuquerque, do Depto de Medicina Tropical/UFPE. Após as apresentações, a Profa. Dra. Vera Lúcia de Menezes Lima convidou o aluno para a apresentação de sua dissertação intitulada: "**Efeito do Tratamento com Derivados Imidazolidínicos 3-benzil-5-(4- cloro-arilazo)-4-tioxo-imidazolidin-2-ona (LPSF-PT5), 3-(4-cloro-benzil)-5(4-nitro-benzilideno)-imidazolidina-2, 4-diona (LPSF-FZ4) e Praziquantel sobre os Lipídeos de Camundongos Infectados por *Schistosoma mansoni*",** e informou que de acordo com o Regimento Interno do Curso, o candidato 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. O aluno procedeu à explanação e comentários acerca do tema em **35 (trinta e cinco) minutos**. Após a apresentação do mestrando, a Sra. Presidente convidou a Banca Examinadora para ocupar seus lugares e passou a palavra a primeira examinadora, Profa. Dra. Mônica Camelo Pessôa de Azevedo Albuquerque que agradeceu o convite, fez alguns comentários e sugestões, e iniciou sua argüição. Ao final, a referida professora deu-se por satisfeita. Em seguida, a Sra. Presidente passou a palavra para a Profa. Dra. Patrícia Maria Guedes Paiva, que agradeceu o convite, fez alguns comentários e sugestões, e iniciou sua argüição. Ao final, a referida professora deu-se por satisfeita. Logo após, a Sra. Presidente passou a palavra para a Profa. Dra. Luana Cassandra Breitenbach Barroso Coelho, que agradeceu ao convite, fez alguns comentários e sugestões, iniciando sua argüição. Ao final, a referida professora deu-se por satisfeita. Em seguida, a Sra. Presidente usou da palavra, na qualidade de orientadora, para tecer alguns comentários, agradecer à Banca Examinadora e parabenizar o candidato. Dando prosseguimento, a sessão foi suspensa para proceder ao julgamento pela Banca Examinadora, a qual se reuniu na Secretaria do Curso. Após alguns comentários, a Banca decidiu, por unanimidade, conceder a menção "**Aprovado com Distinção**". Nada mais havendo a tratar, lavrei a presente ata que vai assinada por mim, Secretário, e demais membros da Banca Examinadora. Recife, 25 de fevereiro de 2008.

Mônica C. P. A. Albuquerque

Patrícia de Guedes Paiva

Vera Lúcia M. Lima

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## RESUMO

A Esquistossomose mansônica é a segunda parasitose humana mais prevalente no mundo, sendo no Brasil um problema de saúde pública. A esquistossomose altera o metabolismo lipídico, promovendo diminuição nos níveis de colesterol total (CT), fosfolipídios totais (FT) e triglicerídeos (TG) plasmáticos em hospedeiros como o camundongo. O praziquantel (PZQ) é o principal agente esquistossomicida empregado para combate à doença. Entretanto, o aparecimento de cepas de *Schistosoma mansoni* resistentes ao tratamento convencional, demonstra a necessidade de desenvolvimento de novas drogas esquistossomicidas. Nesse contexto, as imidazolidinas vêm se destacando como fármacos com notória atividade antiparasitária frente ao *S. mansoni in vitro* e *in vivo*. Este trabalho teve por objetivo avaliar o efeito do tratamento, nas doses de 50mg/kg/dia e 100mg/kg/dia durante 5 dias, com derivados imidazolidínicos 3-benzil-5-(4-cloro-arilazo)-4-tioxo-imidazolidin-2-ona (LPSF-PT5), 3-(4-cloro-benzil)-5-(4nitro-benzilideno)-imidazolidina-2,4-diona (LPSF-FZ4) e PZQ sobre os lipídios plasmáticos de camundongos infectados e não infectados por *S. Mansoni*. Os resultados mostraram que em camundongos infectados por *S. mansoni,* o tratamento com 100mg/kg/dia de PZQ e LPSF-PT5 reduziu significativamente os níveis plasmáticos de CT em 24.59% e 18.60% respectivamente, bem como os níveis de TG em 31.60% e 31.50%, respectivamente, fenômeno explicável pelo efeito da esquistossomose no animal. Com 50 mg/kg/dia e 100 mg/kg/dia, LPSF-FZ4 promoveu respectivamente, 44.80% e 40.30% de redução nos TG plasmáticos de animais infectados. Já nos animais não infectados, somente foi observada com LPSF-FZ4 diminuição nos TG em 26.7% quando tratados com 50 mg/kg/dia e em 21.7% quando tratados com 100 mg/kg/dia. Portanto, analisando as alterações *in vivo* nos lipídios plasmáticos, o derivado imidazolidínico LPSF-FZ4 representou o fármaco com melhor potencial hipotrigliceridêmico.



Descritores: esquistossomose mansônica, colesterol total, atividade hipotrigliceridemia, praziquantel, derivados imidazolidínicos.

## ABSTRACT

The Schistosomiasis mansoni is the second human parasitosis more prevalent in the world, being in Brazil a problem of public health. The schistosomiasis alters lipid metabolism, promoting decrease in plasma levels of total cholesterol (TC), total phospholipids (TF) and triglycerides (TG). The praziquantel (PZQ) is the principal agent schistosomicidal employed for the treatment. However, the apparition of resistant strains to the conventional treatment encourage the development of new anti-schistosomal drugs. In this context, the imidazolidines comes detaching like drugs with potential anti-schistosomal activity front of *S. mansoni in vitro* and *in vivo*. This work aimed to evaluated the effect of treatment, at concentrations of 50mg/kg/day and 100mg/kg/day of imidazolidine derivatives 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5), 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) and PZQ on lipid metabolism of schistosomiasis mansoni infected and uninfected mice. The results showed that in infected mice, treatment with 100mg/kg/day PZQ and LPSF-PT5 significantly reduced plasma TC levels by 24.59% and by 18.60%, respectively as well TG level by 31.60% e 31.50%, respectively fact possibly justified for schistosomiasis effect in the animal. At dose of 50 mg/kg/day and 100 mg/kg/day, LPSF-FZ4 induced significant decrease by 44.80% and by 40.30% on TG levels from *S. mansoni* mice, respectively. On the other hand, uninfected animals treated with 50 mg/kg/day and 100 mg/kg/day LPSF-FZ4 presented about 26.9% and 21.7% significant decreases on plasma TG level. Therefore, analyzing the alterations *in vivo* of plasma lipids evaluated, imidazolidine derivative LPSF-FZ4 represented the compound with better potential hypotrigliceridemic.



Keywords**:** schistosomiasis mansoni, total cholesterol, hipotrygliceridemic activity, praziquantel, imidazolidines derivatives

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## 1. INTRODUÇÃO



## 4. CAPÍTULO I

# SUMÁRIO

# 1. INTRODUÇÃO

No ano de 1851, na cidade do Cairo, Egito, Theodor Maximilian Bilharz, anatomista e helmintologista alemão, descreveu o aparecimento do primeiro esquistossômulo em humanos, caracterizado como um trematoda, inicialmente descrito como *Distomum haematobium* e, hoje *Schistosoma haemotobium.* Ao mesmo tempo, no Japão, outros cientistas também descreveram o aparecimento de outro trematoda, o *Schistosoma japonicum.* Até 1906 apenas tinha-se conhecimento dessas duas espécies, com suas morfologias descritas. Contudo, em 1907, na Inglaterra, observou-se, ocasionalmente, na urina de pacientes com esquistossomose, o aparecimento de ovos com espículos laterais, os quais não se tinham descrição. Luigi Sambon, da Escola de Medicina Tropical de Londres, tinha descoberto outro trematoda denominado *Schistosoma mansoni* (COON, 2005).

A esquistossomose é uma parasitose causada por helmintos do gênero *Schistosoma.* Estima-se que 200 milhões de pessoas em todo o mundo estejam infectadas pelas diferentes espécies do parasito (BLANCHARD, 2004). Destes, 120 milhões são pacientes sintomáticos da fase aguda e 20 milhões são portadores da fase mais severa da doença (CHITSULO *et al*., 2000). De acordo com ENGELS *et al*. (2002), ela é endêmica em 76 países e territórios.

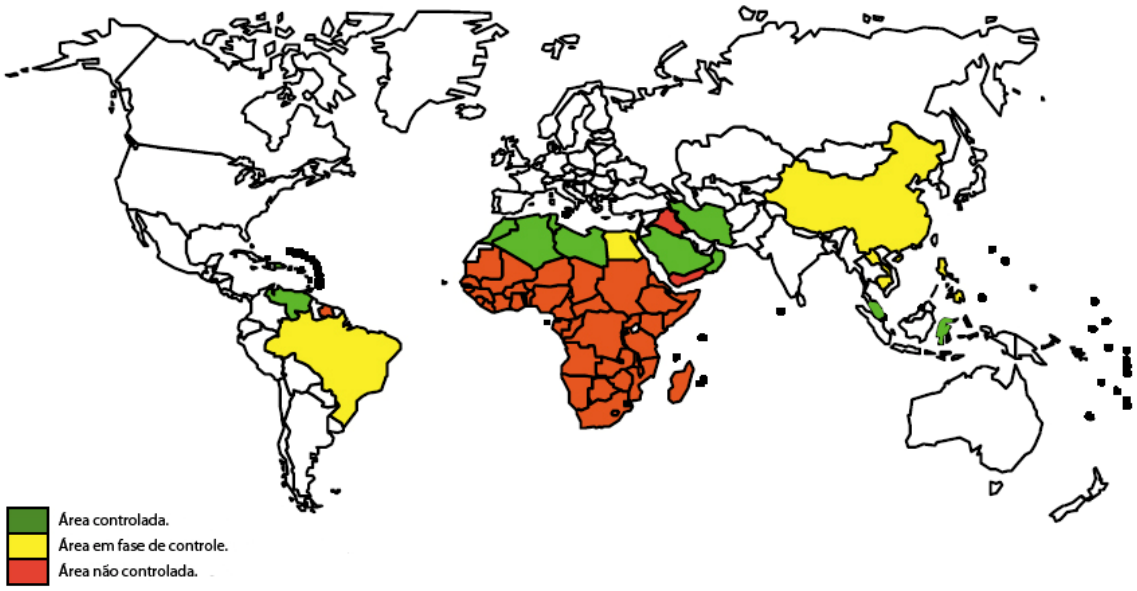


**Figura 1**. Estado de controle da esquistossomose no mundo (ENGELS *et al*., 2002)

O gênero *Schistosoma* é um trematoda da família Schistosomatidae. Este gênero é formado por 4 grupos de espécies, conforme apresentado na tabela 1, sendo que apenas as espécies *S. bovis*, *S. indicum* e *S. nasale* não são parasitas em humanos (COON, 2005).

**Tabela 1**. Grupo de espécies de Schistosoma (COON, 2005)

| Grupos | Espécies |
|---|---|
| *Schistosoma mansoni* | *S. mansoni* |
| *Schistosoma japonicum* | *S. japonicum*<br>*S. mekongi*<br>*S. malayensis* |
| *Schistosoma haematobium* | *S. haematobium*<br>*S. intercalatum*<br>*S. mattheei*<br>*S. bovis* |
| *Schistosoma indicum* | *S. indicum*<br>*S. nasale* |

O *S. mansoni* é a espécie que apresenta a maior distribuição global (JOHNSTON, 1993). Na América do Sul, esta espécie é a causadora desta parasitose (figura 2), distribuindo-se desde países do caribe até vários estados do Brasil (GRYSEELS *et al*., 2006).

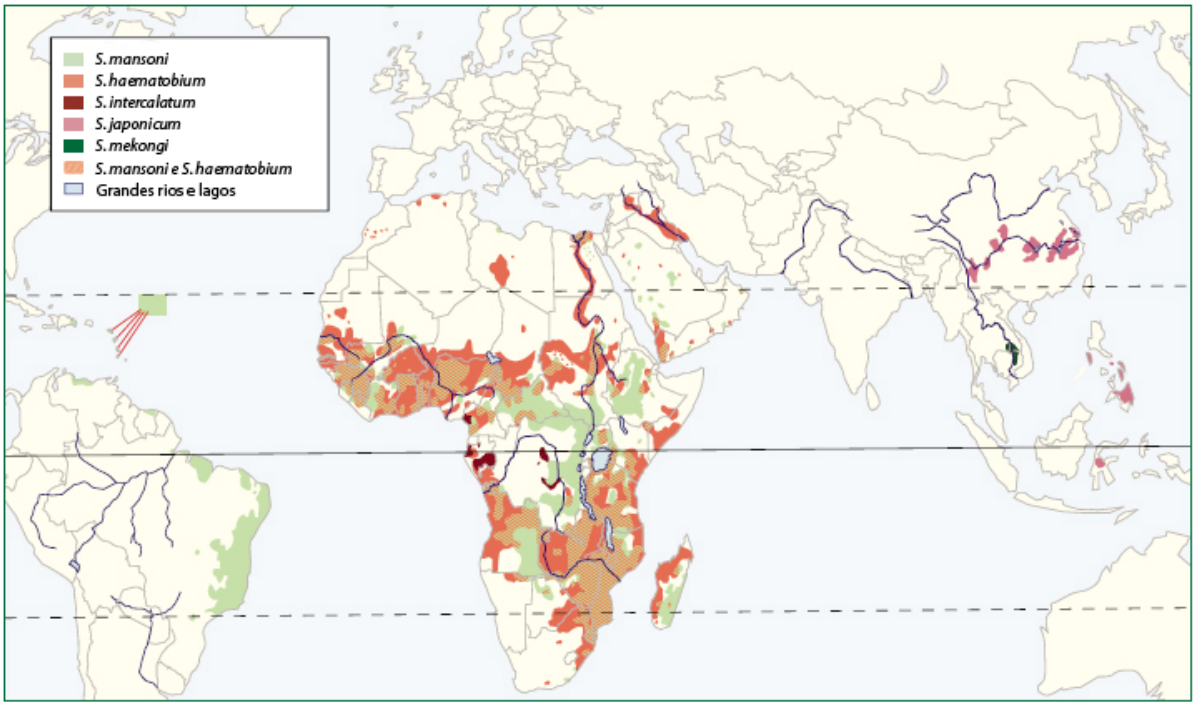


**Figura 2**. Distribuição global do *S. mansoni* (GRYSEELS *et al*., 2006)

Em termos de morbidade, a esquistossomose tem papel de destaque no ranking das parasitoses de importância em saúde pública (WHO, 2005), perdendo apenas para a malária como causa de morbidade em termos de doença parasitária (CHITSULO *et al*., 2000). A nível de Brasil, é estimado que 8 a 10 milhões de pessoas sejam afetadas por esta doença (FERRARI *et al*., 2003). Dados oficiais mostram que no ano de 2006 foram confirmados 7.890 casos dessa doença a nível nacional (BRASIL, 2006). A nível estadual, Pernambuco possui importante prevalência da esquistossomose (BARBOSA *et al.,* 1996). Dados de 1994 mostraram que Pernambuco possuía uma área endêmica de 17.215 km$^2$, o que corresponde a 17,5% de sua área territorial, possuindo 47% de municípios afetados. (MARQUES, 1994).

A esquistossomose é uma parasitose, cujo agente etiológico possui um ciclo evolutivo do tipo heteroxênico, necessitando de dois hospedeiros para desenvolvimento de seu ciclo. O molusco do gênero Biomphalaria destaca-se como hospedeiro intermediário

para esta doença (REY *et al*., 1991). No Brasil, são encontradas três espécies desse molusco que estão envolvidas com a transmissão da doença: *Biomphalaria glabrata, Biomphalaria straminea e Biomphalaria tenagophila* (PARAENSE, 1983). Em termos de distribuição, o *B. glabrata* abrange 16 estados (Alagoas, Bahia, Espírito Santo, Goiás, Maranhão, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rio de Janeiro, São Paulo e Sergipe) e o Distrito Federal. O *B. straminea* tem distribuição conhecida mais extensa, estando presente, praticamente, em todas as bacias hidrográficas do território brasileiro. Ocorre em 23 estados (Acre, Alagoas, Amazonas, Bahia, Ceará, Espírito Santo, Goiás, Maranhão, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Pará, Paraíba, Paraná, Pernambuco, Piauí, Rio Grande do Norte, Rio Grande do Sul, Rio de Janeiro, São Paulo, Santa Catarina, Sergipe e Tocantins) e no Distrito Federal. O *B. tenagophila* é amplamente encontrada no sul do país, embora possa ser detectada em menor extensão em outras regiões. Hoje, sua distribuição alcança 11 estados (Bahia, Goiás, Mato Grosso, Mato Grosso do Sul, Espírito Santo, Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Rio Grande do Sul e Santa Catarina) e o Distrito Federal (FUNASA, 2002). Destas três espécies, o *B. glabrata* é o que apresenta a maior susceptibilidade à infecção pelo *S. mansoni,* devido ao seu maior tamanho, melhor adaptação ao parasita, maior sobrevida e maior numero de larvas infectantes liberadas (MALAGUEÑO *et al*., 1994).

Já o homem, é comumente o hospedeiro definitivo da doença, embora haja, em modelo experimental, o camundongo como excelente hospedeiro. Tudo começa na água, quando as fezes humanas contaminadas com ovos do *S. mansoni* liberam o miracídio (figura 3).

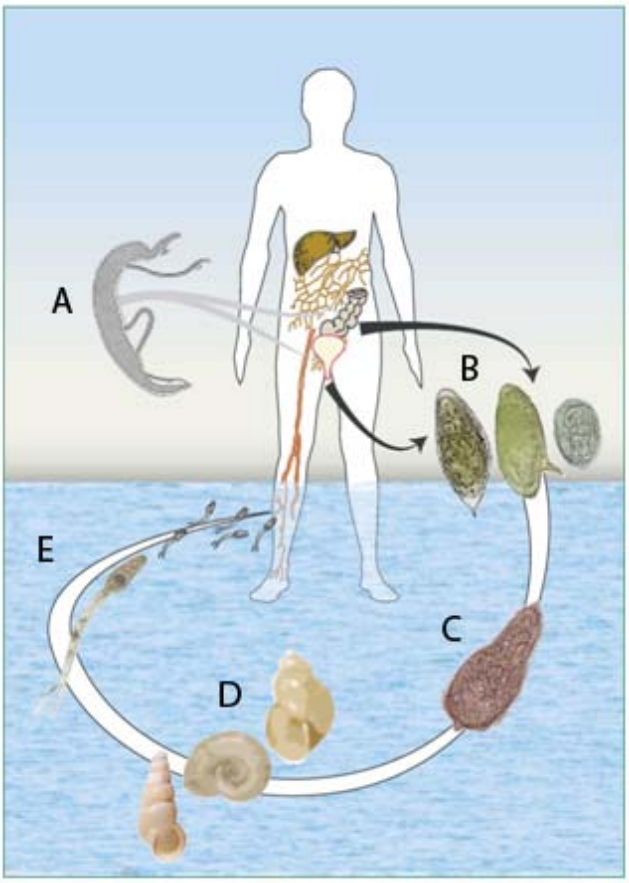


**Figura 3** – Ciclo de vida do *Schistosoma mansoni* (GRYSEELS *et al*., 2006).

A forma larvária (figura 3 - C), o miracídio, existente no interior do ovo, em condições adequadas de luz e temperatura, liberta-se e nada ativamente ao encontro de seu hospedeiro intermediário. Neste (figura 3 - D), ocorre o desenvolvimento dos esporocistos originando as cercárias. Sob estímulos como luz, temperatura, os moluscos liberam várias cercarias (figura 3 - E), as quais nadam ativamente até encontrar seu hospedeiro definitivo. Após transpassar a pele do homem, transformam-se em esquistossômulos os quais são levados pela corrente sanguínea até o pulmão (local de alongamento). Através das veias pulmonares saem do pulmão até ganharem a grande circulação onde são conduzidos ao sistema portal-hepático (figura 3 - A). No interior dos vasos do sistema porta (figura 4), o verme evolui até tornar-se adulto. Após crescimento, ocorre o acasalamento, período que o casal migra para o sistema porta-mesentérico para oviposição.

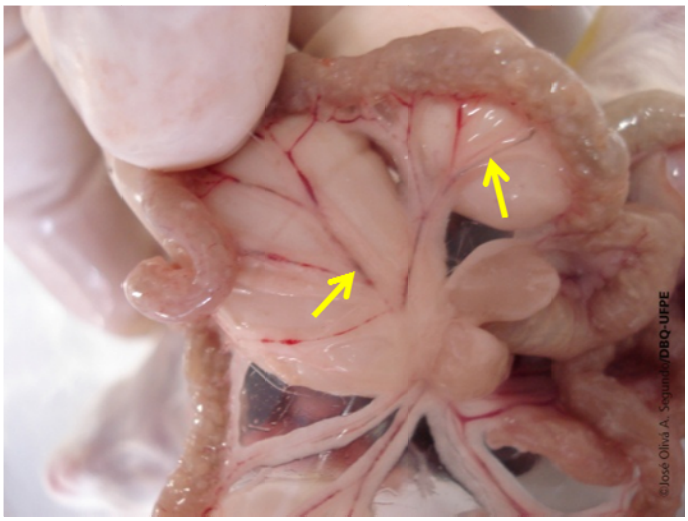


**Figura 4** – Plexo mesentérico de camundongos infectados por *S. mansoni*.

A evolução e a sintomatologia desta parasitose dependem de fatores como espécie do parasito, carga parasitária, freqüência de reinfecções, idade e estado imunológico do indivídio. (KABATEREINE *et al*., 1999). Durante o processo de oviposição, 50% dos ovos eliminados chegam à luz intestinal para serem eliminados junto com as fezes. Entretanto, os ovos que não conseguem chegar à luz intestinal são transportados via circulação, ficando retidos e depositados em órgãos como pulmão, intestino e principalmente o fígado, local das maiores alterações histopatológicas, fisiológicas e bioquímicas (COUTINHO, 1973).

Com a deposição de ovos no fígado, dá-se início à reação inflamatória visando isolar e reter os ovos maduros com a resultante formação de lesões granulomatosas peri-ovulares de localização portal, peri-portal ou parenquimatosa decorrentes da resposta imunológica aos antígenos liberados pelos ovos com conseqüente formação de fibrose hepática (COELHO, 1971). Estas áreas fibrosadas (figura 5) ao longo dos vasos hepáticos podem levar a uma fibrose perivascular, conhecida como fibrose de Symmers, a qual desencadeará outros fenômenos como a hipertensão portal e esplenomegalia (BOGLIOLO, 1957).

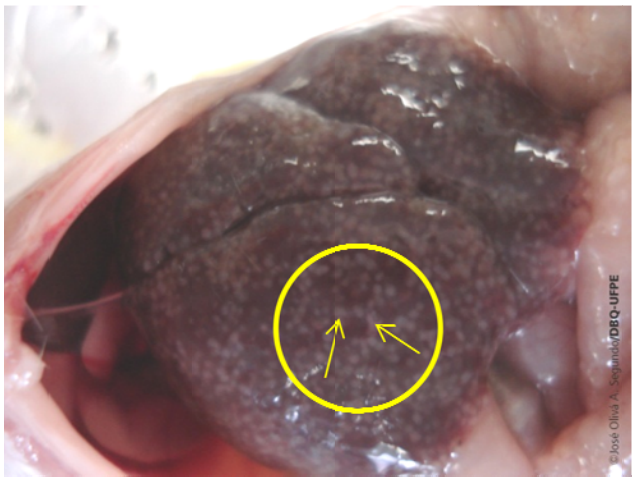

**Figura 5** – Fígado contendo fibroses (pontos brancos) decorrentes de lesões granulomatosas oriundas da infecção por *S. mansoni*

A reação granulomatosa que se desenvolve ao redor dos ovos do parasito, tem como base uma hipersensibilidade do tipo celular (WARREN *et al*., 1967), em decorrência da eliminação de antígenos do miracídio, chamados de SEA (*Soluble egg antigens* – Antígenos solúveis do ovo) que atravessam a casca do ovo, estimulando células como macrófagos, eosinófilos, linfócitos e citocinas como a interleucina-2 e interferon gama (HANG *et al*., 1974; WYLER *et al*., 1978), além também de componentes da resposta imune humoral (DESSEIN *et al.,* 1992; WEBSTER *et al.*, 1996). Contudo, as alterações hepáticas parecem não decorrer apenas do processo granulomatoso e da fibrose formada posteriormente, mas também da liberação de substâncias hepatotóxicas produzidas pelos ovos do parasito (AMIRI *et al*., 1992).

Por conta das alterações fisiopatológicas provocadas principalmente pelo granuloma, alterações na função hepática podem ser detectadas através das dosagens da atividade de enzimas como aspartato aminotransferase (AST) e alanina aminotransferase (ALT), enzimas intracelulares que são liberadas na circulação em algumas hepatopatias, como a

esquistossomose (MANSOUR *et al*., 1982). Estas servem para avaliar a função hepática e conseqüentemente o grau da lesão.

Diversos medicamentos já foram desenvolvidos e utilizados para o combate ao verme (CIOLI *et al*., 1995). Atualmente, por não existir uma vacina contra a doença, por não existir um controle efetivo do vetor e pelas ações sanitárias ineficientes de saúde pública, o tratamento quimioterápico é a principal ferramenta no controle da esquistossomose (CAFFREY, 2007).

Dentre esses medicamentos utilizados, destaca-se o praziquantel, droga de escolha para o tratamento da esquistossomose por apresentar alta eficácia contra todas as espécies do Schistosoma, poucos efeitos adversos e administração em dose única por via oral (DOENHOFF *et al*., 2006).

O praziquantel (PZQ) é um derivado pirazino-isoquinolona, de nome químico 2-ciclohexilcarbonil-1,2,3,6,7,11b-hexa-hidro-4H-pirazino-[2,1-a]isoquinolin-4-ona. Sua estrutura química é apresentada na figura 6.

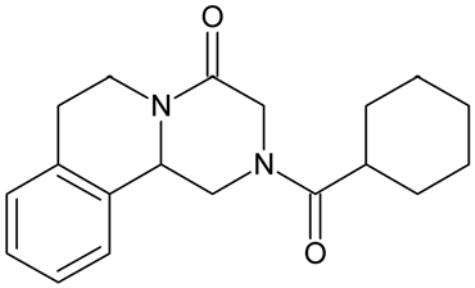


**Figura 6** – Estrutura química do praziquantel.

O mecanismo de ação do PZQ ainda não está totalmente elucidado. Uma das hipóteses aceitas é que o PZQ, ao entrar em contato com verme, cause um influxo de íons

cálcio, levando a um aumento da atividade muscular com conseqüente contração da musculatura, levando a uma ruptura do tegumento helmíntico (PAX *et al*., 1978; FETTERER *et al*., 1980; BECKER *et al*., 1980). O PZQ apresentou baixa toxicidade e mostrou-se seguro e efetivo em sua administração (CIOLI *et al*., 2003). Porém, ele apresenta alguns efeitos secundários mínimos como dor de cabeça, desconforto abdominal (DAVIS, 1993), possuindo uma alta taxa de eficácia (DAYAN, 2003).

Outra alternativa medicamentosa pouco empregada para o controle da doença é o oxaminiquine, cuja ação no verme é inibir a síntese de seus ácidos nucléicos, diminuindo a motilidade do parasito (OLDS *et al*., 2000). Porém, ao contrário do PZQ, essa droga mostra-se eficaz somente para a espécie *S. mansoni* (BECK *et al*., 2001) e apresenta maior relação custo/tratamento em comparação ao PZQ que custa de 7 a 19 centavos de US$ o comprimido de 600 mg (McFADYEN, 2006) .

Entretanto, alguns trabalhos vêm mostrando o aparecimento de cepas de *S. mansoni* resistentes ao tratamento convencional. Produzida em laboratório uma mistura de gênes de *S. mansoni* oriundas de quatro diferentes áreas geográficas, foi verificado que 93% dos vermes resistiram ao tratamento em 3 doses de 300mg/kg de PZQ (FALLON *et al*., 1994). Em outro estudo epidemiológico, foi verificado que em uma área do Senegal, país africano cuja prevalência da doença era de 91% na população, o tratamento convencional com PZQ apresentou baixa taxa de eficácia de tratamento (18%) (STELMA *et al*., 1995). Portanto, é necessário realizar estudos mais criteriosos para verificar outros locais de cepas resistentes ao tratamento. Embora existam outras drogas alternativas como a oxaminiquina para pronta ação, tem sido incentivados estudos visando o desenvolvimento de novos compostos

com o intuito de implementar novas drogas esquistossomicidas (UTZINGER *et al*., 2003; XIAO *et al*., 2007; MOREIRA *et al*., 2007).

Nesse contexto, as imidazolidinas vêm se destacando como fármacos com boa atividade antiparasitária frente ao *S. mansoni in vitro* e *in vivo* (PITTA *et al.*, 2006; ALBUQUERQUE *et al*., 2005; OLIVEIRA *et al.*, 2004).

As imidazolidinas são compostos heterocíclicos pentagonais aromáticos e apresentam em sua estrutura dois átomos de nitrogênio (figura 7) (BARREIRO *et al*., 2001).

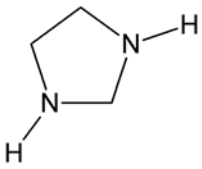


**Figura 7** – Estrutura química do anel imidazolidínico.

Esses compostos, assim como seus derivados, demonstraram possuir várias atividades biológicas, dentre as principais como anticonvulsivante (THENMOZHIYAL *et al*., 2004), antimicrobiana (LIMA *et al*., 1992), atividade anti-hipertensiva (DYLAG *et al*., 2004), atividade antineoplásica (ROBINSON *et al*., 2001), além de atividade esquistossomicida já citada anteriormente.

Através de reações de síntese orgânica, é possível a adição de outros grupamentos químicos ao anel imidazolidínico. Ao adicionar dois grupamentos cetônicos nas posições 2/4 do anel (figura 8), obtém-se como produto a imidazolidina-2,4-diona ou também chamada de Hidantoína.

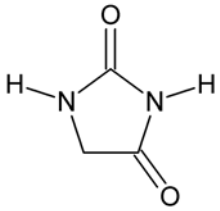

**Figura 8** - Estrutura química da imidazolidina-2,4-diona

Entretanto, ao adicionar-se um grupamento tioxo na posição 4 e um grupamento cetônico na posição 2 do anel, obtemos a 4-tioxo-imidazolidin-2-ona (figura 9).

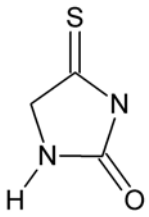

**Figura 9** – Estrutura química da 4-Tioxo-imidazolidin-2-ona

O derivado 3-(4-cloro-benzil)-5-(4nitro-benzilideno)-imidazolidina-2,4-diona (LPSF-FZ4) (figura 10), cuja síntese é descrita por Lima *et al*. (2002), é obtido através da reação do brometo de benzila ou fenacila com a imidazolidina-2,4-diona**,** dando origem ao composto intermediário imidazolidina-2,4-diona alquilado, o qual reage com o ácido acético/acetato de sódio e benzaldeído aromático, levando a formação do derivado LPSF-FZ4, cuja algumas características químicas estão indicadas na tabela 2. Este composto mostrou-se efetivo contra vermes do *S. mansoni in vitro,* apresentando uma taxa de mortalidade de 100% no 9$^{o}$ dia de contado a uma concentração de 60 μg/mL (ALBUQUERQUE, 2002).

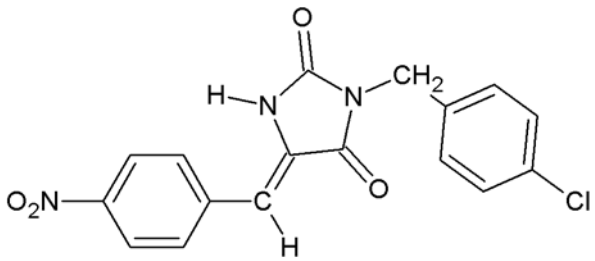


**Figura 10** – Estrutura química do 3-(4-cloro-benzil)-5-(4nitro-benzilideno)-imidazolidina-2,4-diona (LPSF-FZ4).

Outro derivado, o 3-benzil-5-(4-cloro-arilazo)-4-tioxo-imidazolidin-2-ona (LPSF-PT5) (figura 11), cuja síntese é descrita por Brandão *et al*. (1997), é produzido através de três reações. A 3-benzil-imidazolidina-2,4-diona, obtida através da reação da imidazolidina-2,4-diona com o clereto de benzil reage com $P_2S_5$, formando 3-benzil-4-tioxo-imidazolidin-2-ona que reage com a anilina substituída em meio ácido e na presença de nitrato de sódio, dando origem a formação de compostos arilazo-imidazolidiônicos, dentre eles o LPSF-PT5, cujas algumas características químicas estão indicadas na tabela 2. Foi verificado que, através de ensaios de susceptibilidade *in vitro* frente ao *S. mansoni* (cepa BH), mostrou possuir relevante atividade esquistossomicida, causando letalidade em 100% dos vermes a partir do quarto dia após o início do tratamento a uma concentração entre 20 a 60 µg/mL (SOARES, 2004).

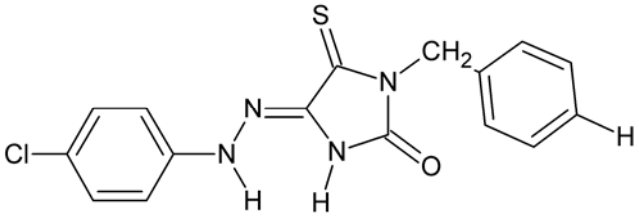


**Figura 11** – Estrutura química do 3-benzil-5-(4-cloro-arilazo)-4-tioxo-imidazolidin-2-ona (LPSF-PT5)

**Tabela 2 –** Algumas características químicas dos compostos 3-(4-cloro-benzil)-5-(4nitro-benzilideno)-imidazolidina-2,4-diona (LPSF-FZ4) e 3-benzil-5-(4-cloro-arilazo)-4-tioxo-imidazolidin-2-ona (LPSF-PT5).

| Composto | Cor | Fórmula | Massa (mols) |
|---|---|---|---|
| LPSF-FZ4 | Amarelo | $C_{17}H_{12}N_3O_4Cl$ | 357,7 |
| LPSF-PT5 | Alaranjado | $C_{16}H_{12}N_4OSCl$ | 343,5 |

Os lipídios são biocompostos com diversos grupamentos químicos, cuja principal característica é sua insolubilidade em água e solubilidade em solventes orgânicos (LEHNINGER *et al*., 2002). Os lipídios presentes no plasma, mais importante do ponto de vista fisiológico e clínico, são os ácidos graxos, fosfolipídios que são componentes das membranas biológicas, triglicerídeos (TG) que servem de armazenamento energético mais importante do organismo (presente principalmente no tecido adiposo), e colesterol (precursor dos hormônios esteróides, dos ácidos biliares, da Vitamina D além de exercer importantes funções nas membranas biológicas) (GRAUBER, 2000). Esses lipídios são transportados de um órgão para outro, utilizando o plasma sanguíneo como rede de transporte, principalmente na forma de lipoproteínas, molécula composta por lipídios e proteínas específicas (apoproteinas), tendo como exemplos principais: o quilomicron, a lipoproteína de muito baixa densidade (VLDL), a lipoproteína de baixa densidade (LDL) e a lipoproteína de alta densidade (HDL).

Estudos anteriores demonstraram que a esquistossomose promove diversas alterações no metabolismo lipídico como diminuição nos níveis de colesterol total (CT), fosfolipídios e TG plasmáticos em humanos (COUTINHO-ABATH *et al*., 1966) (GILLETT *et al*., 1978), em camundongos (OWEN *et al*., 1978; VIEIRA *et al.*, 1992) e em primatas não humanos da espécie *Callithrix jacchus* (LIMA *et al*., 1998), na fase crônica da doença. Hipóteses tais como a não produção de colesterol pelo schistosoma (MEYER *et al*., 1970),

incorporação de partículas de LDL pelo parasita (BENNETT *et al*., 1991) via internalização de receptores LDL (RUMJANEK *et al*., 1983), indução da síntese de anticorpos naturais que metabolizam o colesterol através de opsonização (ALVING *et al*., 1999) e também diminuição de atividade da enzima esterificante do colesterol na circulação, a lecitina: colesterol aciltransferase (LCAT) (LIMA *et al*., 1998) justificam a diminuição desses lipídios.

A literatura pouco reporta sobre alterações promovidas por drogas esquistossomicidas sobre os lipídios plasmáticos. O PZQ, ao interagir com a bicamada lipídica das membranas biológicas promove uma discreta esfoliação de alguns lipídios constituintes da mesma, diminuindo discretamente o colesterol e fosfolipídios da membrana de eritrócito humano (MALHEIROS *et al*., 2000).

Derivados imidazólicos, análogos estruturais das imidazolidinas que se diferenciam destas por possuir 4 átomos de hidrogênio a menos (figura 12), muitos desses empregados em terapias antimicrobianas, mostraram possuir atividade sobre o metabolismo lipídico (WIERZBICKI, 2003).

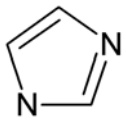


**Figura 12** – Estrutura do anel imidazólico.

Dentre esses, podemos destacar o metronidazol, muitas vezes empregado para o combate de amebíase. Este fármaco promove, em humanos, diminuições significativas de 19% de colesterol LDL e de 23% de triglicerídeos, bem como discreto aumento de 10% nos níveis de colesterol HDL (SHAMKHANY *et al*., 2003). Outros trabalhos mostraram que o cetoconazol, agente antimicótico, também possui potencial atividade hipolipidêmica, promovendo as

mesmas ações supracitadas no metabolismo lipídico, porém em percentuais menores (10% de redução de LDL colesterol e discreta ação redutora sobre triglicerídeos) (KRAEMER *et al*., 1986; STALENHOEF *et al*., 1997). Além disso, derivados imidazolicodinônicos promoveram alterações positivas, elevando o nível de colesterol HDL (ELOKDAH *et al*., 2000). Em geral, os derivados imidazólicos promovem inibição das enzimas do citocromo P450, incluindo aquelas que estão envolvidas com a esterificação do colesterol, como a acil-CoA colesterol acil transferase (ACAT) (AHMED *et al*., 1995), bem como promovem aumento de atividade de receptores LDL (BURNETT *et al*., 1999; KEMPEN *et al*., 1987).

Também é reportado na literatura que alguns compostos imidazolidínicos demonstraram possuir alguma atividade que promovessem alterações no metabolismo lipídico. Foi observado que a fenitoína, cujo nome químico é 5,5-difenil-imidazolidina-2,4-diona, empregada para tratamento de epilepsia, quando administrada por via oral em cavalos, promoveu elevação dos TG bem como alteração a utilização e incorporação de ácidos graxos (FLETCHER, *et al*., 1993), elevação das concentrações plasmáticas de HDL-C em humanos (GOERDT *et al*., 1995) e, independentemente do nível de HDL-C, reduziu o aparecimento precoce e o desenvolvimento da aterosclerose (TROCHO *et al*., 2004). A Nitrofurantoína, de nome químico 1-(5-nitro-2-furfurilideneamino)-hidantoina, utilizada para o tratamento de infecções do trato gênito-urinário, promoveu inibição da peroxidação lipídica microssomal em ratos (DUBIN *et al*., 1987). Também a Clonidina (n-(2,6-diclorofenil)-4,5-dihidro-1H-imidazol-2-amina), utilizada como anti-hipertensivo, causou em pacientes com hipertensão leve uma diminuição de HDL-C e de apolipoproteína A-I e apolipoproteína A-II (HOUSTON *et al.*, 1990).

Como a esquistossomose promove diminuição nos lipídios plasmáticos como CT, TG e FLT e também, devido existir poucos trabalhos mostrando o efeito de fármacos com ação esquistossomicida sobre os lipídios plasmáticos, estudos que visem observar o efeito do tratamento antiparasitário sobre o metabolismo lipídico são de extrema importância, uma vez que mudanças que promovam elevações, principalmente, nos níveis de CT e TG, estejam relacionadas com o aparecimento e desenvolvimento das doenças cardiovasculares, apontadas hoje como um grave problema de saúde pública, acarretando em milhares de óbitos em todo mundo. Drogas que demonstrem possuir ação no metabolismo lipídico, como alguns derivados imidazólicos, análogos estruturais das imidazolidinas, estão sendo apontados como potenciais coadjuvantes para terapias hipolipidêmicas. Por isso, torna-se não só importante o estudo dos derivados imidazolidínicos frente ao *S. mansoni*, mas também em relação ao metabolismo de lipídios e lipoproteínas visando elucidar novas drogas com potencial atividade hipolipidêmica.

# 2. OBJETIVOS

## 2.1. Geral

Avaliar o efeito do tratamento com os derivados imidazolidínicos 3-benzil-5-(4-cloro-arilazo)-4-tioxo-imidazolidin-2-ona (LPSF-PT5), 3-(4-cloro-benzil)-5-(4nitro-benzilideno)-imidazolidina-2,4-diona (LPSF-FZ4) e do Praziquantel sobre o metabolismo lipídico de camundongos infectados e não infectados por *S. mansoni*.

## 2.2. Específicos

- Infectar camundongos fêmeas com o *S. mansoni* (cepa BH);
- Tratar via oral, por 5 dias consecutivos, camundongos infectados e não infectados com os derivados imidazolidínicos LPSF-PT5 e LPSF-FZ4 e Praziquantel nas concentrações de 50 mg/kg/dia e 100 mg/kg/dia.
- Determinar os níveis dos lipídios plasmáticos (CT, TG e FT) e atividade enzimática de AST e ALT dos camundongos infectados e não infectados antes e após 15 dias do tratamento com os compostos avaliados.
- Avaliar estatisticamente os resultados dos parâmetros bioquímicos obtidos entre os grupos de estudo.

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# 4. CAPÍTULO I - EFFECT OF TREATMENT WITH NEW IMIDAZOLIDINE DERIVATIVES AND PRAZIQUANTEL ON PLASMA LIPID OF MICE INFECTED BY *SCHISTOSOMA MANSONI*

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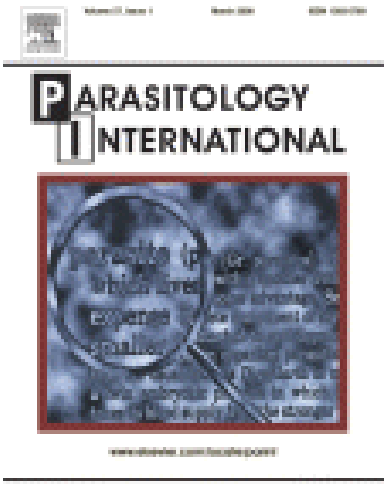

**Effect of treatment with new imidazolidine derivatives and praziquantel on plasma lipid of mice infected by *Schistosoma mansoni***

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Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; CMC, Carboxymethylcellulose; TC, total cholesterol; TG, triglycerides; PZQ, praziquentel.

### 4.1. Abstract

The Schistosomiasis mansoni is responsible for an elevated mortality in world. The parasitosis promotes modifications in lipid metabolism such as low levels of plasma total cholesterol (TC) triglycerides (TG) and total phospholipids (TPL) and it is not reported alterations on plasma lipids induced by anti-schistosomal drugs. Imidazolic compounds are analogous structural of imidazolidines and presents hypolipidemic activity. In this work, it was evaluated the effect on plasma lipids of oral treatment for 5 days with new imidazolidine compounds 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5), 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) and Praziquantel (PZQ) at concentration of 50 mg/kg/day and 100 mg/kg/day with *Schistosoma mansoni* mice and also uninfected mice. PZQ and LPSF-PT5 significantly decrease by 24.59% and by 18.60% TC levels, respectively as well by 31.60%, 31.50% TG levels, respectively from plasma of infected mice treated only at 100 mg/kg/day. In uninfected groups it was not observed a significant reduction on lipids evaluated. LPSF-FZ4 at concentration of 50 mg/kg/day and 100 mg/kg/day promoted significant reductions about 44.80% and about 40.30%, respectively only on plasma TG levels from *S. mansoni* mice. Nevertheless, in both concentrations, it was observed that LPSF-FZ4 significantly decreased plasma TG level by 26.7% and by 21.7% from uninfected animals. The results suggest that similar to PZQ treatment with 50mg/kg/day and 100mg/kg/day, LPSF-PT5 does not affect the plasma CT, TG and TPL when treated for 5 days. On the other hand, LPSF-FZ4 in both evaluated doses showed to alter TG levels, candidate as a potential hypotrigliceridemic drug.

**Keywords:** schistosomiasis mansoni, total cholesterol, hipotrygliceridemic activity, praziquantel, imidazolidines derivatives

## 4.2. Introduction

Schistosomiasis mansoni is the second human parasitosis more prevalent in the world [1] which affect about 200 million of people [2]. In Brazil, it is estimated about 8 to 10 million of individual is in affected by *S. mansoni* [3], the more difunded specie [4]. By depositing eggs of parasites in the liver, place of the larger histopathologic, physiological and biochemistry alterations, the worm leads to formation of granulomatous lesions [5], promoting alterations in the hepatic function, detected by intracellular enzyme activity assay as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [6]. The diseases induces alterations on lipid metabolism such as decrease in plasma total cholesterol (TC) and triglycerides (TG) [7], low levels of plasma total phospholipids (TPL) in humans [8], mice [9] [10] and on non-human primates of specie *Callithrix jacchus* (sagui) [11].

Due to absence of vaccines against worms, chemotherapy is the mainly alternative employed for the treatment [12]. Praziquantel (PZQ) is the principal drug active against all schistosome species by single oral dose administration [13]. Nevertheless, some *S. mansoni* strains are resistant to the conventional treatment [14] [15], encouraging the development of new anti-schistosomal drugs [16] [17]. Imidazolidines are aromatic pentagonal heterocycle compounds [18] which have several biological activities such as anticonvulsivant [19], antihypertensive [20], anticancerous [21] and anti-schistosomal activity for *S. mansoni* [22] [23] [24].

It is not reported alterations on plasma lipids induced by anti-schistosomal drugs. Metronidazol and other imidazolic derivatives which are analogous structural of imidazolidines, used in antimicrobiotic therapies, present activity hypolipidemic [25] in humans [26]. Some common used imidazolidines compounds has activity on lipid and lipoproteins metabolisms such as fenitoin [27] [28] [29], nitrofurantoin [30] and clonidine [31].

This work shows the effects of treatment with the new imidazolidine derivatives 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) and 3-(4-chloro-benzyl)-

5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) as well as with Praziquantel on lipid metabolism of schistosomiasis mansoni infected mice.

## 4.3. Materials and Methods

### 4.3.1. Drugs

The compounds 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) and 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) (Figure 1) were synthesized at the LPSF (Laboratório de Planejamento e Síntese de Fármacos, Departamento de Antibióticos, UFPE, Recife, Brazil). The synthesis and physical chemistry characters from compounds has been described by Lima et al.(1992) [32] and Brandão et al. (1997) [33], respectively. Praziquantel (PZQ) from Sigma© was utilized for this work.

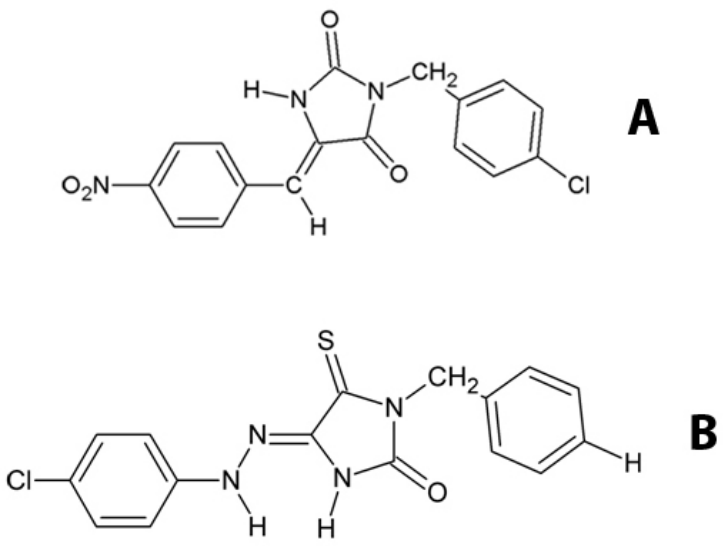


**Figure 1**

### 4.3.2. Animals

Female Swiss albino mice, 25 days old were kept under standard environmental conditions (22 ± 2 °C; 12/12 h light/dark cycle). The animals were fed with a standard diet (Labina Purina®, Brazil) and water *ad libitum*. The experimental procedure was approved by the Local Committee for Animal Ethics (Protocol 137/2003-CEEA/CCB).

4.3.3. Animal infection

The infection was carried out by tail immersion technique according to the method of Duvall and Dewitt (1967) [34] in mice of 25 days old, through the exposure to 80±10 cercariae/mouse of a BH (BH – Belo Horizonte, Brazil) strain of *S. mansoni*. The infected mice were kept under standard conditions for 49 days before start treatment.

4.3.4. Animal groups and treatment

Groups (n = 8) of infected and uninfected mice, by experimental antiparasitary treatment were orally treated during 5 days with 50mg/kg/day and 100mg/kg/day LPSF-PT5, LPSF-FZ4 and PZQ solubilized in 0.5% (w/v) carboxymethylcellulose (CMC) (from Sigma©). As control, group of infected and uninfected mice received oral CMC for the same period. Therefore, animals were kept under standard conditions for 15 days after treatment, sacrificed on the next day. In uninfected groups, treatment started at 75 days old.

4.3.5. Blood collection

Fasting blood collection was collected before treatment and after 15 days one. Blood (250µl) was taken from the retro-orbital sinus with a capillary utilizing brief ether anesthesia, anticoagulated with EDTA (1mg/ml) and kept in ice before using. All blood samples were centrifuged at 2,500 *x g* at 4$^{o}$C for 18 minutes to separate erythrocytes and plasma which was used for the experiments.

4.3.6. Biochemical parameters analysis

Plasma TC and TG levels were measured by enzymatic colorimetric methods (Labtest Diagnostica, Brazil/SA). Plasma lipid extract was obtained according manufacturer instructions kit for measure TPL (Labtest Diagnostica, Brazil/SA). AST and ALT activities were determined by enzymatic colorimetric methods (Labtest Diagnostica, Brazil/SA).

4.3.7. Statistical analysis

Values are expressed as means ± S.E.M. For the comparison between infected and uninfected mice groups before treatment it was used unpaired t test. For investigation of effect of treatment, before and after 15 days, in infected and uninfected mice groups for each concentration used was utilized Two-way ANOVA. It was used 4.0 version of GraphPad Prism computer software for analysis. Significant differences are suitable for values of probability $p < 0.05$, $p < 0.01$ and $p < 0.001$.

## 4.4. Results

4.4.1. Plasma Lipids

50 days after *S. mansoni* infection, mice presented significant ($p < 0.001$) low plasma TC levels when compared to uninfected mice (table 1) before treatment. Table 2 shows the comparison between plasma TC levels before and after 15 days of treatment with analyzed compounds. The results shown that in infected mice, the treatment with 100mg/kg/day of PZQ and LPSF-PT5 induced a significant decrease in plasma TC levels by 24.59% ($p < 0.001$) and by 18.60% ($p < 0.01$), respectively. All the three drugs did not significantly decrease TC levels plasma of uninfected mice.

Schistosomiasis mansonic induced significant ($p < 0.001$) decrease on plasma TG levels when compared to uninfected animals before treatment (table 1). In infected mice, treatment with 100mg/kg/day of PZQ, LPSF-PT5 and LPSF-FZ4 promoted significant decrease about 31.60% ($p < 0.01$), 31.50% ($p < 0.01$) and 40.30% ($p < 0.001$), respectively as well about 44.80% ($p < 0.001$) when treated with 50mg/kg/day of LPSF-FZ4 on plasma TG levels (table 3). However, only 50 mg/kg/day and 100mg/kg/day LPSF-FZ4 promoted significantly decreasing on plasma TG levels by 26.7% ($p < 0.05$) and by 21.7% ($p < 0.05$) , respectively in uninfected mice.

Furthermore, in this work, the treatment with all evaluated compounds at dose of 50mg/kg/day and 100mg/kg/day did not promote alterations on plasma TPL levels. The infected mice TFL levels were slightly decreased in comparison to uninfected mice before treatment (table 1).

Groups which received CMC not significantly decrease TC, TG and TPL plasma levels.

#### 4.4.2. Enzyme activities

*S. mansoni* infected mice presented significant ($p < 0.001$) rise AST and ALT activities in relation to uninfected mice before treatment (table 1). The infected and uninfected groups treated with 100 mg/kg/day PZQ demonstrated a significant increases by 40.92% ($p < 0.01$) and by 37.80% ($p < 0.01$) respectively in AST enzyme activity (table 4). Treatment with imidazolidines derivatives LPSF-PT5 and LPSF-FZ4 as well CMC not promoted significantly modifications in the AST enzyme activity.

Treatment with all evaluated compounds in both concentrations did not promote significantly alterations on ALT enzyme activity (table 5).

### 4.5. Discussion

#### 4.5.1. Biochemical alterations

According to various factors such as parasite species, parasitic load, frequency of reinfections, age and individual immunological state [35], the inflammatory reaction caused by egg deposit in liver results in the formation of fibrosis which throughout the vase can lead to the perivascular fibrosis known as fibrosis of the Symmers which will take another phenomenons like portal hypertension and esplenomegaly [36]. In this work, *S. mansoni* infected mice presented elevated AST and ALT enzyme activities, indicating hepatic dysfunction. This result is in agreement with hepatic parameters found in plasma of mice with *S. mansoni* [37].

It has been reported that infection by *S. mansoni* alters plasma lipid levels. The low levels of plasma TC found in present work has several possibilities such as not production of cholesterol by schistosomes [38], incorporation of low density lipoprotein (LDL) by the parasite [39] via inducible LDL receptors [40], induction of synthesis of natural antibodies that metabolize cholesterol by opsonization [41] as also reduction of the enzyme activity of lecitin: cholesterol acyltransferase (LCAT) [42] which catalyse the esterification of cholesterol on plasma. Carbohydrate metabolism is affected by *S. mansoni* infection by decreased assimilation of glucose in humans [43] such as hypoglicemy in mice [44] which causes greater mobilization of TG stored, leading to decreases ones. This justify the low plasma TG level found. Increased plasma lipid levels such as TC and TG are associated to

development of heart diseases. However, effects produced by schistosomiasis on lipid metabolism promote a significant reduction in development of atherosclerotic lesions from *S. mansoni* infected mice [45]. Therefore, it is necessary to evaluate the effects produced by conventional and new anti-schistosomal drugs on plasma lipids.

4.5.2. Effects produced by treatment

Still today, PZQ is the main clinical alternative against schistosomiais. This drug interacts with lipid layer of biological membranes and promotes a discrete exfoliation of some lipids constituent of eritrocit layer human, decreasing slight the cholesterol and phospholipids [46]. In this work, treatment with 100mg/kg/day PZQ promoted significant decrease on plasma TC and TG from *S. mansoni* infected mice. However, the sum of TC reduction percentual from infected control mice, plus slight TC reduction percentual from uninfected group treated with 100mg/kg/day PZQ is practically equal to TC reduction percentual found. Similarly, the significant TG reduction percentual found in infected animals treated with 100mg/kg/day PZQ is justified by same reasoning. Therefore, 100mg/kg/day PZQ treatment for 5 days not induced alterations on plasma lipids analyzed. But, maybe if the 500 mg/kg PZQ treatment was carried out for a more prolonged time, for exemple 10 days, plasma TC and TG reduction percentuals obtained were bigger because PZQ effect is not dependent on the maximum drug concentration to which site is exposed, but rather on the length of time during which site is exposed to a threshold drug concentration [47]. Even if AST activity was significantly increased (table 4), 100 mg/kg/day PZQ treatment used in this work was not demonstrated to alter hepatic function. Probable there was discrete toxical action of PZQ in other organs whose AST concentrations is higher than in liver, such as heart, esqueletic muscle, spleen and kidney. Therefore, it is necessary specific studies to evaluate this possibility.

Some Imidazolic derivatives acts by inibiting P450 enzymes, including those involved with cholesterol esterification such as acyl-CoA cholesterol acyl transferase (ACAT) [48] and by enhance LDL receptor activity [49] [50]. The mechanism of action of imidazolidines is not well established. At example of the hypothesis placed for PZQ, results with 100mg/kg/day LPSF-PT5 points for a probable hypocholesterolemic and hypotrigliceridemic actions, since that treatment be prolonged because treatment with 100 mg/kg/day 2-substituted sulfanyl-

3,5-dihydro-imidazole-4-one derivatives for 8 days promoted significant changes in serum lipids levels from rats [51]. Furthermore studies are requested for investigate this hypotheses. Nevertheless, according to our results, LPSF-FZ4 did not reduce TC levels from plasma of uninfected animals in none of the doses evaluated, although was observed a slight TC reduction on plasma from infected group treated with 50 mg/kg/day and 100 mg/kg/day LPSF-FZ4. These decreases obted is due for *S. mansoni* infection (table 2). On the other hand, results with 50 mg/kg/day LPSF-FZ4 showed a significant diminution on plasma TG from infected and uninfected mice after 15 of treatment. Significant plasma TG reductions obted with 50 mg/kg/day and 100mg/kg/day LPSF-FZ4 (table 3) from infected mice probably is not only due to infection by *S. mansoni* but too by effect of treatment. This indicates a potential hypotrigliceridemic action, requesting further confirmation. Treatment with compounds LPSF-PT5 and LPSF-FZ4 in both concentrations did not promote significantly alterations on AST and ALT enzyme activities. This found shows that treatment does not modify hepatic function in both groups of infected and uninfected mice even observed a slight reduction of AST and ALT activities at 50 mg/kg/day (table 4-5). This indicates a good animal tolerability in treatment with these compounds.

In conclusion, new imidazolidine compounds LPSF-FZ4 and LPSF-PT5 such as PZQ demonstrated to low plasma lipids levels. It is request furthermore studies for better comprehension of effects on lipid metabolism. LPSF-FZ4 showed to be a potential candidate for hypotrigliceridemic therapy who could be aim at combat cardiovascular diseases which are main causes of death in world [52].

### 4.6. Acknowledgements

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### 4.8. Tables

**Table 1** - Comparison of biochemical parameters from uninfected and infected mice groups before treatment.

| Parameters | Groups | | Significance |
|---|---|---|---|
| | Uninfected | Infected | |
| Total Cholesterol (mmol/l) | 2.14 ± 0.04 | 1.75 ± 0.03 | $p < 0.001$ |
| Triglycerides (mmol/l) | 1.48 ± 0.05 | 1.12 ± 0.03 | $p < 0.001$ |
| Total Phospholipids (mmol/l) | 12.60 ± 0.26 | 12.10 ± 0.25 | $p > 0.05$ |
| Aspartate Aminotransferase (U/l) | 32.43 ± 0.79 | 45.41 ± 1.67 | $p < 0.001$ |
| Alanine Aminotransferase (U/l) | 15.21 ± 0.35 | 24.75 ± 1.34 | $p < 0.001$ |

Statistical significance determined by unpaired t test. Data are stated as means ± S.E.M

**Table 2** - Plasma Total Cholesterol levels before and after 15 days of treatment with praziquantel (PZQ), 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) and 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) in schistosomiasis mansoni infected and uninfected mice.

| Groups (n = 8) | Concentration (mg/kg/day) | Plasma Total Cholesterol (mmol/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CMC | | PZQ | | LPSF-PT5 | | LPSF-FZ4 | |
| | | Before | After | Before | After | Before | After | Before | After |
| Infected | 50 | 1.81 ± 0.10 | 1.61 ± 0.09 | 1.74 ± 0.09 | 1.58 ± 0.07 | 1.79 ± 0.06 | 1.55 ± 0.09 | 1.73 ± 0.12 | 1.49 ± 0.08 |
| Infected | 100 | 1.74 ± 0.05 | 1.55 ± 0.07 | 1.83 ± 0.05 | 1.38 ± 0.06*** | 1.72 ± 0.09 | 1.40 ± 0.05** | 1.70 ± 0.07 | 1.58 ± 0.05 |
| Uninfected | 50 | 2.19 ± 0.12 | 2.19 ± 0.08 | 2.22 ± 0.18 | 2.25 ± 0.30 | 2.25 ± 0.12 | 2.22 ± 0.07 | 2.28 ± 0.11 | 2.26 ± 0.11 |
| Uninfected | 100 | 2.05 ± 0.13 | 2.10 ± 0.18 | 2.00 ± 0.07 | 1.75 ± 0.05 | 2.09 ± 0.06 | 1.92 ± 0.05 | 2.02 ± 0.09 | 2.08 ± 0.08 |

Data are stated as means ± S.E.M.**p<0.01; ***p<0.001 when compared simultaneously all compounds (Two-way ANOVA followed by Bonfferroni test) before and after treatment for each concentration.

**Table 3** - Plasma triglycerides levels before and after 15 days of treatment with praziquantel (PZQ), 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) and 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) in schistosomiasis mansoni infected and uninfected mice.

| Groups (n = 8) | Concentration (mg/kg/day) | Plasma Triglycerides (mmol/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CMC | | PZQ | | LPSF-PT5 | | LPSF-FZ4 | |
| | | Before | After | Before | After | Before | After | Before | After |
| Infected | 50 | 1.14 ± 0.08 | 0.95 ± 0.07 | 1.02 ± 0.11 | 0.86 ± 0.10 | 1.14 ± 0.12 | 0.86 ± 0.09 | 1.07 ± 0.10 | 0.59 ± 0.04 *** |
| Infected | 100 | 1.13 ± 0.06 | 0.91 ± 0.07 | 1.20 ± 0.04 | 0.82 ± 0.10** | 1.11 ± 0.02 | 0.76 ± 0.05** | 1.14 ± 0.11 | 0.68 ± 0.05*** |
| Uninfected | 50 | 1.37 ± 0.12 | 1.45 ± 0.30 | 1.35 ± 0.22 | 1.01 ± 0.10 | 1.66 ± 0.21 | 1.42 ± 0.16 | 1.53 ± 0.20 | 1.12 ± 0.06* |
| Uninfected | 100 | 1.50 ± 0.11 | 1.59 ± 0.15 | 1.57 ± 0.13 | 1.16 ± 0.16 | 1.51 ± 0.09 | 1.32 ± 0.15 | 1.52 ± 0.14 | 1.19 ± 0.06* |

Data are stated as means ± S.E.M.. *p < 0.05; **p<0.01; ***p<0.001 when compared simultaneously all compounds (Two-way ANOVA followed by Bonfferroni test) before and after treatment for each concentration.

**Table 4** - Plasma aspartate aminotransferase activity before and after 15 days of treatment with praziquantel (PZQ), 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) and 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) in schistosomiasis mansoni infected and uninfected mice.

| Groups (n = 8) | Concentration (mg/kg/day) | Aspartate Aminotransferase (U/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CMC | | PZQ | | LPSF-PT5 | | LPSF-FZ4 | |
| | | Before | After | Before | After | Before | After | Before | After |
| Infected | 50 | 39.56 ± 1.72 | 35.66 ± 2.80 | 41.25 ± 2.30 | 35.85 ± 1.83 | 41.10 ± 1.67 | 36.23 ± 2.86 | 43.74 ± 1.82 | 38.33 ± 2.24 |
| Infected | 100 | 51.47 ± 2.25 | 49.15 ± 2.19 | 56.62 ± 2.66 | 79.79 ± 1.85** | 54.58 ± 2.06 | 59.42 ± 2.26 | 42.08 ± 2.46 | 36.93 ± 1.63 |
| Uninfected | 50 | 31.26 ± 1.96 | 32.36 ± 2.11 | 31.56 ± 2.37 | 27.97 ± 1.46 | 31.71 ± 1.85 | 25.98 ± 2.66 | 30.08 ± 1.93 | 26.66 ± 2.69 |
| Uninfected | 100 | 34.76 ± 2.08 | 33.75 ± 2.47 | 37.49 ± 1.66 | 51.66 ± 2.55** | 30.84 ± 2.04 | 34.46 ± 2.06 | 33.60 ± 2.03 | 28.10 ± 2.02 |

Data are stated as means ± S.E.M. **p<0.01 when compared simultaneously all compounds (Two-way ANOVA followed by Bonfferroni test) before and after treatment for each concentration.

**Table 5** - Plasma alanine aminotransferase activities before and after 15 days of treatment with praziquantel (PZQ), 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) and 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) in schistosomiasis mansoni infected and uninfected mice.

| Groups (n = 8) | Concentration (mg/kg/day) | Alanine Aminotransferase(U/l) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | CMC | | PZQ | | LPSF-PT5 | | LPSF-FZ4 | |
| | | Before | After | Before | After | Before | After | Before | After |
| Infected | 50 | 23.06 ± 2.58 | 23.59 ± 2.62 | 22.17 ± 1.97 | 18.91 ± 1.56 | 25.57 ± 2.66 | 19.10 ± 2.56 | 26.82 ± 1.99 | 21.24 ± 2.84 |
| Infected | 100 | 20.43 ± 2.87 | 20.71 ± 2.07 | 18.80 ± 1.72 | 21.27 ± 2.61 | 21.55 ± 2.26 | 23.52 ± 1.76 | 25.08 ± 1.79 | 20.99 ± 2.87 |
| Uninfected | 50 | 14.58 ± 1.11 | 13.00 ± 0.75 | 14.95 ± 0.72 | 12.32 ± 0.89 | 15.28 ± 0.97 | 11.31 ± 0.84 | 14.04 ± 0.96 | 10.45 ± 0.68 |
| Uninfected | 100 | 16.21 ± 0.46 | 15.50 ± 0.41 | 15.51 ± 1.20 | 16.93 ± 1.38 | 15.79 ± 1.28 | 15.02 ± 1.17 | 14.68 ± 0.70 | 12.36 ± 1.62 |

Data are stated as means ± S.E.M.

### 4.9. Figure legends

**Figure 1** – Chemical structure of compounds, 3-(4-chloro-benzyl)-5-(4nitro-benzylidene)-imidazolidine-2,4-dione (LPSF-FZ4) (A) and 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one (LPSF-PT5) (B).

# 5. CONCLUSÕES

I. A infecção por *S. mansoni* (Cepa BH) promoveu diminuição nos níveis de colesterol total e triglicerídeos em camundongos. Entretanto não foi observada redução nos níveis de fosfolipídios totais plasmáticos. A função hepática dos camundongos infectados por *S. mansoni* (Cepa BH) mostrou-se alterada, em detrimento das elevações de atividades enzimáticas de aspartato aminotransferase e alanina aminotransferase.

II. O tratamento com o derivado imidazolidínico LPSF-PT5 e Praziquantel, a uma concentração de 100mg/kg/dia promoveu redução significativa de colesterol total e triglicerídeos plasmáticos em camundongos com a esquistossomose além de possuir boa tolerabilidade.

III. O derivado imidazolidínico LPSF-FZ4, a uma concentração de 50 mg/kg/dia e 100 mg/kg/dia, mostrou apresentar potencial atividade hipolipidêmica, reduzindo os triglicerídeos plasmáticos em camundongos infectados e não infectados, além de ser bem tolerado.

# 6. ANEXOS

## 6.1. Normas para redação de artigos para a revista "Parasitology International"

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