Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi: 10.1016/S1995-7645(14)60117-6

# *Aristolochia gehrtii* inhibits liver toxicity and apoptosis in *Schistosoma malayensis* infection

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ARTICLE INFO

*Article history:*
Received 10 May 2014
Received in revised form 15 June 2014
Accepted 15 August 2014
Available online 20 September 2014

*Keywords:*
*Schistosoma malayensis*
*Aristolochia gehrtii*
Aristolochiaceae
Liver
Apoptosis
Mice

ABSTRACT

**Objective:** To evaluate a protective effect of *Aristolochia gehrtii* (*A. gehrtii*) leaves to inhibit liver toxicity and apoptosis in *Schistosoma malayensis* (*S. malayensis*) infection. **Methods:** Forty male albino mice were divided into four equal groups: group 1 control including non-infected healthy mice and groups 2, 3 & 4 subcutaneously infected with *S. malayensis* cercariae where groups 3 & 4 pretreated with *A. gehrtii* leaves (200 mg/kg, bwt) & cinnamoylamide (250 mg/kg, bwt), respectively. **Results:** *S. malayensis* caused a significant increase in serum AST, ALT, ALP, MDA, NO, bilirubin, urea, creatinine, total cholesterol, LDL, triglycerides, and HDL levels. The pretreatment of *A. gehrtii* leaves and cinnamoylamide significantly inhibited that increase. On the other hand, *S. malayensis* induced a significant decrease in serum total protein, albumin, globulin, albumin/globulin ratio, blood SOD and GPx, while *A. gehrtii* leaves and cinnamoylamide pretreatment increased the above parameters. Treatment with *A. gehrtii* leaves and cinnamoylamide to *S. malayensis* infected mice increased p53 expression but decreased bcl-2 expression. These results were supported by histopathological investigations. **Conclusions:** *A. gehrtii* inhibits liver toxicity and apoptosis in *S. malayensis* infection and this effect is associated with the major cinnamoylamide ingredient of *A. gehrtii* leaves.

## 1. Introduction

Schistosomiasis remains one of the most prevalent parasitic infections in the world. It has been estimated that more than 207 million people are infected and 779 million people are at risk of infection[1]. Schistosomiasis, also known as bilharzia, is a parasitic disease that leads to chronic ill health. It is the major health risk in the rural areas of China and South East Asia and continues to rank high in other developing countries[2]. The disease is diagnosed either by the presence of blood in the urine or, in the case of intestinal schistosomiasis, by initially atypical symptoms which can lead to serious complications involving the liver[2]. Schistosomiasis, caused by various *Schistosoma* species, is estimated to cause infection in more than 200 million people in rural agricultural and peri-urban areas in tropical and subtropical developing countries particularly in China, Africa and South America[3,4]. In South East Asia, *Schistosoma japonicum* is prevalent in southern Philippines and Indonesia, whereas *Schistosoma mekongi* is endemic in the Mekong Delta[5,6]. In Malaysia, *Schistosoma malayensis* (*S. malayensis*) were detected among the Orang Asli in west Malaysia, while an evidence of *Schistosoma* was found in a monkey and in liver and rectal biopsies of immigrants from Philippines[7-10]. In Sarawak, environmental impact assessment (EIA) survey in upper Lupar River and upper Rejang River found eight individuals who were active schistosome eggs excretes. A sero-survey found 16.2% were seropositive for schistosomiasis, however the snail host could be identified and the *S. malayensis*-like infection among the Penan and other interior tribes (Orang Ulu) in upper Rejang River Basin was reported[11,12].

The research for a new and natural source to be used as a protection for schistosomiasis was increased in the last decade. The medicinal plants provide a new, available and

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cheap source for developing new drugs nowadays. Natural products account for more than 40% of all pharmaceuticals on the market today, where from 1941 to 2002, over 50% of all the drugs, or new drug entities, available for cancer treatment were derived from natural resources[13].

Aristolochiaceae family is widely distributed in tropical, subtropical and temperate regions of the world. They are known to occur in Asia, Africa, North and South America and Australia. It is represented by three genera: Aristolochia, which consists of approximately 100 species, Holostylis and Euglypha, each of which consists of only one species *Holostylis reniformis* and *Euglypha rojasiana*[14]. The latter have been suggested to be included in the *Aristolochia* genus[15]. With regard to synonyms, more than 300 names have been proposed for these species. The majority of the *Aristolochiales* are tropical, though a number of them range as far north as Canada, Scandinavia, and Northern Japan. *Aristolochia* species are herbaceous perennials, under-shrubs or shrubs, often scandent, scrambling, twining, sometimes lianas, usually with prostrate or tuberous rhizomes or rootstocks, and alternate, pinnate, polymorphic or lobed leaves[16-18]. In traditional medicine, these plants are known as "one thousand men" and have been mainly used as abortifacients, stomachics, antiophidians, antiasthmatics, expectorants, and, recently, in slimming therapies[14].

The selection of *Aristolochia gehrtii* (*A. gehrtii*) leaves for the current study was due to its major bioactive alkamides constituents[19], where amides have antioxidant activity[20]. The purpose of the present study was to discover a new natural adjunctive therapeutic agent to treat *S. malayensis*-like infection complications in liver tissue. To accomplish this, we measured several oxidative stress and apoptosis parameters to determine if *S. malayensis* induces oxidative stress in liver and if this stress could be prevented by the use of *A. gehrtii* leaves extract.

## 2. Materials and methods

### 2.1. Plant material and ethanolic extract

*A. gehrtii* leaves were provided from Penang Botanical Garden, Penang, Malaysia in September 2012. The plant was botanically identified authenticated at School of Pharmaceutical Science, Universiti Sains Malaysia, Malaysia. Voucher specimen of each plant was deposited at the herbarium of the pharmacy school. The leaves were crushed, pulverized and then weighed and prepared for extraction.

*A. gehrtii* leaves (5 kg) were air-dried in an oven at 40 ℃ for 4 d and then the dry leaves was cut and pulverized. Then, they were subjected to size reduction to get coarse powder. The powdered material was subjected to successive extraction in a Soxhlet apparatus using solvent petroleum ether (40-60 ℃) and ethyl alcohol. This powder was vacuum dried in a desiccator's till it was free from moisture[21].

### 2.2. Mice

Forty male albino mice (20-25 g) were obtained from the animal house of the Advanced Medical and Dental Institute, Universiti Sains Malaysia, Malaysia and were kept in special plastic cages. The animals were maintained on a commercial balanced diet and tap water. The experiments were performed after approval from the ethics committee of the Universiti Sains Malaysia and in accordance with recommendations for the proper care and use of laboratory animals (NIH publication no. 85:23 revised 1985).

### 2.3. Estimation of lethal dose 50 ($LD_{50}$) of the extract

Twenty-four mice were orally administered the leaves extract with different four concentrations (1, 3, 5 and 7 mg/kg, bwt). Six rats were kept as control group throughout the entire experimental period. Mortality was assessed and counted in the different groups. $LD_{50}$ was calculated according to the method of Behrens and Karber[22]. Finally, 1/10 of the $LD_{50}$ was found to be safe[23].

### 2.4. Thin layer chromatography (TLC) for isolation of leaves extract alkamide

TLC was used to resolve and isolate the cinnamoylamide constituent from leaves extract. Precoated silica gel plates (20×20 cm, Brinkman Silplate F-22, with fluorescent indicator, 0.25, 0.50, or 2.00 mm gel thickness) were used in all separations. With these plates, the substituted amide present was easily detected as yellow or golden bands by viewing the developed plates under long-wave ultraviolet light. Amide was isolated by applying the extract as bands to TLC plates, developing in appropriate solvent systems, locating the compounds under ultraviolet light, and then scraping and eluting with chloroform or acetone. Compound was crystallized directly from appropriate solvents (usually ether or ether-hexane), or if necessary, the compound was subjected to further cleanup by TLC. The solvent systems used and the numbers used to designate them are as follows: 1, methylene chloride; 2, chloroform-ethyl acetate (2:1, v/v); 3, methylene chloride-ether (2:1, v/v); 4, benzene-ether (20:1, v/v); 5, hexane-ethyl acetate-methanol (5:5:1, v/v); and 6, methylene chloride-ether (1:1, v/v)[19]. This process was taken 2 months from ethanolic extract until isolation of cinnamoylamide powder was obtained. The isolation ratio=1:5 where 60 g of cinnamoylamide ingredient obtained from 300 g of the extract. The dose of cinnamoylamide used in this study was chosen[19].

### 2.5. Test for purity, quality and stability of the extract and cinnamoylamide

Purity tests (microbiological, pesticide residues, heavy metals, radioactive residues, chemical, foreign organic matter and sulfated ash) were performed in accordance with Malaysian accepted protocol requirements and accredited to ISO/IEC 17025 consulting WHO guidelines on stability and quality controls methods for medicinal plants[24,25]. The leaves extract and amide were stored in a tightly cooling (-40 ℃) sealed container away from heat and light that prevented a loss of extract solvent and entry of water; plant extract or amide was dissolved in 1 mL distilled water and given orally to individual rat.

### 2.6. Experimental design

The mice model had been established and divided into four equal (10 mice each) groups as follows: (Ⅰ) Control group including non-infected healthy, parasite free mice as a control group; (Ⅱ) *S. malayensis* infected group represented by subcutaneously infected with 50 cercariae of *S. malayensis*; (Ⅲ) *A. gehrtii* leaves treated group comprising *S. malayensis* infected group pretreated daily with oral dose of *A. gehrtii* leaves extract (200 mg/kg, bwt) for 7 d before *Schistosoma* infection; (Ⅳ) Cinnamoylamide treated group including *S. malayensis* infected group pretreated daily with oral dose of cinnamoylamide (250 mg/kg, bwt) for 7 d before *Schistosoma* infection.

All mice were anesthetized 24 h after the last oral administration by inhalation with diethyl ether solution. All mice were massed at the beginning and the end of the study.

### 2.7. Blood sampling and handling

Blood samples were collected from retro-orbital plexus of mice using capillary tubes[26] into clean centrifuge tubes. Part of blood sample was collected in the presence of using EDTA as an anticoagulant for blood, while the other part of the blood sample was allowed to coagulate and centrifuged at 4 000 r/min for 15 min to separate blood serum which stored at -20 ℃.

### 2.8. Biochemical analysis

To evaluate liver function, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, alkaline phosphatase (ALP), total protein, albumin, globulin and albumin/globulin ratio were determined colorimetically using kits reagents obtained from Biomeriéux Company, France. To detect lipid fractions, serum total cholesterol, low-density lipoproteins (LDL), triglycerides and high-density lipoprotein (HDL) were determined colorimetically using kits reagents also obtained from Biomeriéux Company, France. Antioxidants enzymes including blood superoxide dismutase (SOD), glutathione peroxidase (GPx), serum lipid peroxidation (MDA) and plasma nitrate and nitrite concentrations as an indicator of nitric oxide (NO) generation were determined colorimetically using kits reagents purchased from Randox Company, British.

### 2.9. Immunohistochemical detection of p53 and bcl-2 expression in liver tissue

Immunohistochemical staining procedure for p53 and bcl-2 was performed using the methods described by Hsu and Raine[27] and Elias *et al*[28].

### 2.10. Histopathological examinations

Specimens of liver were fixed in 10% (v/v) neutral formalin solution and then processed for routine technique for embedding in paraffin. Blocks were sectioned at a thickness of 5 $\mu$m and stained with hematoxylin and eosin for histopathological examination, which were performed under light microscopy and documented by an Olympus microphotocamera.

### 2.11. Statistical analysis

The results are expressed as mean ± standard error (SE). Statistical significance was determined through one-way analysis of variance (ANOVA), followed by Student's *t* test. *P* values less than 0.05 were considered statistically significant.

## 3. Results

### 3.1. Phytochemical composition and $LC_{50}$ of the extract

Phytochemical studies of the plant leaves extract showed the presence of six alkamides in ethanolic extract of the plant leaves as a major ingredient. The concentration of each compound were 90.2, 47.4, 50.0, 121.2, 154.8 and 119.8 mg/g extract for alkamides compounds 1, 2, 3, 4, 5 and 6, respectively; so compound 5 (cinnamoylamide) was chosen for such study. Other chemical ingredients of the plant leaves extract included five lignans, three terpenes, two benzoic acid derivatives, and six phenethyl derivatives.

The maximum non-lethal dose was found to be 2 000 mg/kg; hence 1/10 of the dose was taken as effective dose (200 mg/kg, bwt). In the present study, there were no observable differences of body weight, food or drink intake during experimental period of the study.

### 3.2. Liver function

The results presented in Table 1 indicated that *S. malayensis* caused a significant increase ($P<0.05$) in serum AST, ALT,

ALP and bilirubin levels in mice. The prereatment with either *A. gehrtii* leaves extract or cinnamoylamide for 7 d before *S. malayensis* infection inhibited the increase in AST, ALT, ALP and bilirubin as compared to the *S. malayensis*–infected mice.

Table 1 revealed the protective role of *A. gehrtii* leaves extract or cinnamoylamide on serum total protein, albumin, globulin levels, and albumin/globulin ratio in *S. malayensis*–infected mice. It is obvious that *S. malayensis* caused a significant decrease ($P<0.05$) in serum total protein, albumin, globulin levels, and albumin/globulin ratio as compared with control healthy mice. While either *A. gehrtii* leaves extract or cinnamoylamide pretreated for 7 d before *S. malayensis* infection induced a significant increase in total protein albumin, albumin, globulin levels, and albumin/globulin ratio in *S. malayensis*–infected mice as compared with *S. malayensis*–infected mice. On the other hand, *A. gehrtii* leaves extract or cinnamoylamide pretreatment to *S. malayensis*–infected mice revealed an insignificant increase in serum albumin and albumin/globulin ratio while significant increase in serum total protein ($P<0.05$) compared to *S. malayensis*–infected mice group. *S. malayensis*–infected mice + *A. gehrtii* leaves extract or cinnamoylamide exhibited a highly significant increase ($P<0.01$) in serum globulin.

### 3.3. Lipid fractions

The data presented in Table 2 showed that *S. malayensis* induced a significant increase ($P<0.05$) in serum cholesterol on the contrary; *A. gehrtii* leaves extract or cinnamoylamide treatment to *S. malayensis*–infected mice exhibited an insignificant increase in serum cholesterol compared to healthy mice.

The level of serum LDL in normal mice after being infected with *S. malayensis* and then treated with *A. gehrtii* leaves extract or cinnamoylamide was exhibited in Table 2. Analysis of the changes occurred in LDL after *S. malayensis*–infection showed a significant increase ($P<0.05$) in serum LDL, but administration with *A. gehrtii* leaves extract or cinnamoylamide to mice infected with *S. malayensis* revealed an insignificant increase in LDL compared to healthy mice.

Analysis of serum triglycerides in normal, *S. malayensis* infected– or pretreated with either *A. gehrtii* leaves extract or cinnamoylamide to *S. malayensis* infected mice was shown in Table 2. Serum triglycerides after *S. malayensis* infection revealed a significant increase ($P<0.05$) in serum triglycerides, while *A. gehrtii* leaves extract or cinnamoylamide pretreatment to *S. malayensis* infected mice revealed an insignificant increase in serum triglycerides compared to healthy normal mice.

### 3.4. Antioxidants enzymes

It was found that a significant decrease ($P<0.05$) in blood SOD and GPx were recorded in association with *S. malayensis* infection and the data were tabulated in Table 3. On the contrary, the pretreatment with *A. gehrtii* leaves extract or cinnamoylamide to *S. malayensis* infected mice induced insignificant increase in blood SOD and GPx when compared with healthy normal group. On the other hand, *A. gehrtii* leaves extract or cinnamoylamide to *S. malayensis* infected group showed a significant increase ($P<0.05$) in blood SOD but a highly significant increase ($P<0.01$) in blood GPx compared to the *S. malayensis* infected group.

*S. malayensis* caused a significant increase ($P<0.05$) in serum MDA and plasma NO as compared with the normal

**Table 1**
Effect of *A. gehrtii* and cinnamoylamide on liver function of *S. malayensis* infected mice.

| Group | AST (U/L) | ALT (U/L) | Total bilirubin (mg/dL) | ALP (U/L) | Total protein (g/dL) | Albumin (g/dL) | Globulin (g/dL) | Albumin/ Globulin ratio |
|---|---|---|---|---|---|---|---|---|
| Control | 123.1±3.8 | 62.5±2.5 | 0.56±0.08 | 210.5±6.8 | 7.64±0.72 | 3.76±0.45 | 3.88±0.25 | 0.97±0.05 |
| *S. malayensis* | 140.2±0.7$^{*}$ | 82.0±2.0$^{**}$ | 0.74±0.05$^{*}$ | 241.2±7.1$^{**}$ | 6.10±0.35$^{*}$ | 2.81±0.17$^{*}$ | 3.29±0.17$^{*}$ | 0.85±0.03$^{*}$ |
| *A. gehrtii*+*S. malayensis* | 126.4±4.2 | 60.1±2.9$^{a}$ | 0.58±0.04$^{a}$ | 215.1±6.6$^{b}$ | 8.16±0.93$^{a}$ | 3.96±0.48$^{a}$ | 4.18±0.18$^{b}$ | 0.95±0.04$^{a}$ |
| Cinnamoylamide+*S. malayensis* | 128.3±3.9 | 65.0±2.9$^{a}$ | 0.60±0.07$^{a}$ | 223.0±4.9$^{a}$ | 7.75±0.68$^{a}$ | 3.86±0.65 | 3.89±0.25 | 0.90±0.07 |

Data presented as mean±SE. Number of animals=10 per group. $^{*}$Significant change ($P<0.05$) compared to control healthy mice. $^{**}$Highly significant change ($P<0.01$) compared to control healthy mice. $^{a}$Significant change ($P<0.05$) compared to *S. malayensis* group. $^{b}$Highly significant change ($P<0.01$) compared to *S. malayensis* group.

**Table 2**
Effect of *A. gehrtii* and cinnamoylamide on lipid fractions of *S. malayensis* infected mice (mg/dL).

| Group | Cholesterol | LDL | Triglycerides | HDL |
|---|---|---|---|---|
| Control | 98.2±3.5 | 40.7±1.9 | 47.1±2.0 | 48.1±1.9 |
| *S. malayensis* group | 107.5±2.9$^{*}$ | 46.1±1.9$^{*}$ | 52.4±1.9$^{*}$ | 50.9±2.0 |
| *A. gehrtii*+*S. malayensis* group | 101.4±4.1$^{a}$ | 43.5±2.0 | 50.3±2.1 | 47.8±1.9 |
| Cinnamoylamide+*S. malayensis* group | 99.5±3.9 | 41.8±1.7$^{a}$ | 48.2±1.7$^{a}$ | 47.6±1.7 |

Data presented as mean±SE. Number of animals=10 per group. $^{*}$Significant change ($P<0.05$) compared to control healthy mice. $^{a}$Significant change ($P<0.05$) compared to *S. malayensis* group.

healthy mice. A significant decrease ($P<0.05$) was observed in serum MDA and plasma NO in either group pretreated with *A. gehrtii* leaves extract or cinnamoylamide before *S. malayensis* infection as compared with those infected with *S. malayensis* (Table 3).

### 3.5. Anti-apoptotic activity

Figure 1 shows the photomicrographs of immunohistochemically stained liver tissue sections revealing that saline administration induced normal amount of expressed p53 (Figure 1A). On the other hand, the amount of p53 was potentially decreased in *S. malayensis* infected mice (Figure 1B) as compared to control healthy mice. The pretreatment of *S. malayensis* infected mice with *A. gehrtii* leaves extract or cinnamoylamide increased the amount of expressed p53 (Figure 1C & 1D) as compared to *S. malayensis* infected mice.

Figure 2 exhibits the photomicrographs of immunohistochemically stained liver tissue sections revealing that normal mice showed normal amount of expressed bcl-2 (Figure 2A). On the other side, the amount of bcl-2 was potentially increased in *S. malayensis* infected mice (Figure 2B). The treatment of *S. malayensis* infected mice with either dose of *A. gehrtii* leaves extract or cinnamoylamide potentially decreased the elevated amount of expressed bcl-2 (Figure 2C & 2D) as compared to *S. malayensis* infected mice.

### 3.6. Histology study

Figure 3 reveals the histopathology results in *S. malayensis* infected mice as well as *A. gehrtii* leaves extract or cinnamoylamide potentially pretreated groups. The structure

**Table 3**
Effect of *A. gehrtii* and cinnamoylamide on antioxidant idexes of *S. malayensis* infected mice.

| Group | SOD (U/mL) | GPx (U/L) | MDA ( $\mu$ mol/L) | NO ( $\mu$ mol/L) |
|---|---|---|---|---|
| Control | 260.0±8.4 | 6 250±83 | 3.51±0.26 | 35.8±1.8 |
| *S. malayensis* group | 236.9±7.9$^{*}$ | 5 985±99$^{*}$ | 5.76±0.54$^{**}$ | 50.1±2.3$^{**}$ |
| *A. gehrtii*+*S. malayensis* group | 258.7±8.6$^{a}$ | 6 240±87$^{a}$ | 3.56±0.38$^{b}$ | 37.0±2.0$^{b}$ |
| Cinnamoylamide+*S. malayensis* group | 265.1±7.9$^{a}$ | 6 435±100$^{b}$ | 3.71±0.42$^{b}$ | 40.5±1.9$^{a}$ |

Data presented as mean±SE. Number of animals=10 per group. $^{*}$Significant change ($P<0.05$) compared to control healthy mice. $^{**}$Highly significant change ($P<0.01$) compared to control healthy mice. $^{a}$Significant change ($P<0.05$) compared to *S. malayensis* group. $^{b}$Highly significant change ($P<0.01$) compared to *S. malayensis* group.

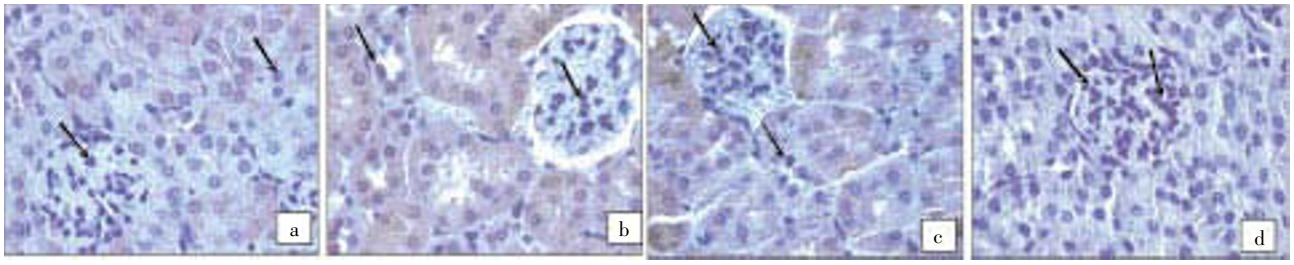


**Figure 1.** *p53* expression in control, *S. malayensis*-infected, cinnamoylamide and *A. gehrtii* extract pretreated mice.
(a) Immunohistochemically-stained liver tissue sections showing apoptotic marker, *p53* expression in control mice. There is almost negligible expression of *p53* in the liver sections of control group. (b) The tissue sections showing the effect of *S. malayensis* on apoptotic marker *p53* expression. *S. malayensis* decreased strongly *p53* expression in liver sections. (c) There was partial expression of *p53* as evidenced by weak immuno-staining in the mice liver treated with cinnamoylamide. (d) The expression of *p53* was increased markedly on administration of *A. gehrtii* leaves extract which was well evident by the positive staining.

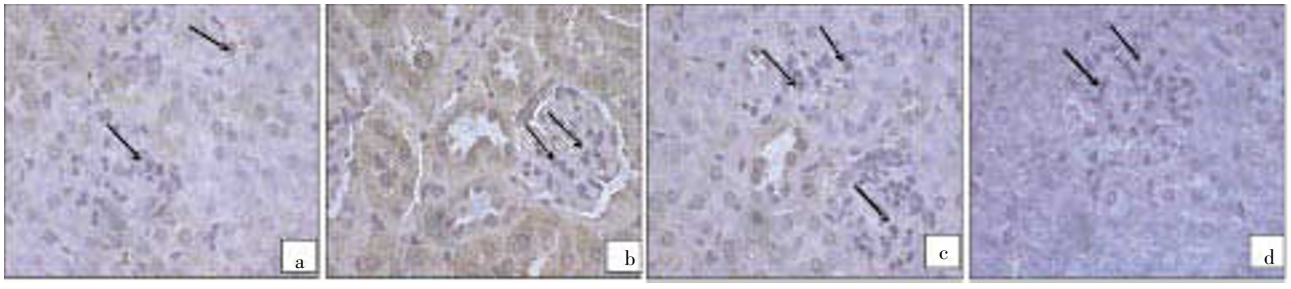


**Figure 2.** *bcl-2* expression in control, *S. malayensis*-infected, cinnamoylamide and *A. gehrtii* extract pretreated mice.
(a) Immunohistochemically-stained kidney tissue sections showing *bcl-2* expression in control healthy group which almost shows normal amount of expressed *bcl-2*. (b) *S. malayensis* strongly induced *bcl-2* expression in liver sections. (c) There is partial inhibition of *bcl-2* expression as evidenced by weak immune-staining in the mice liver treated with cinnamoylamide. (d) *A. gehrtii* leaves extract treatment showed lesser expression which implies that *bcl-2* has been inhibited.

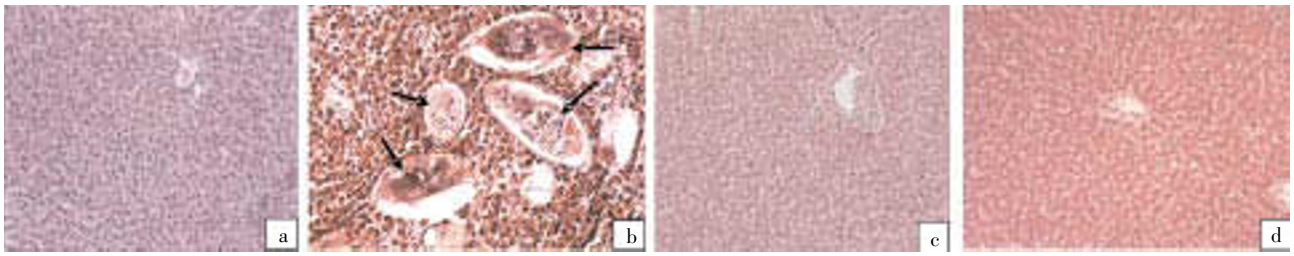


**Figure 3.** Liver histology in control, *S. malayensis*-infected, cinnamoylamide and *A. gehrtii* extract pretreated mice.
(a) Shows the control group with preserved hepatic architecture (H&E, ×200). (b) Shows *S. malayensis* liver granuloma with ova embedded in dense fibrous tissue (arrows). The thin-walled, non-striated helminth ova are not operculated and contain nonvital miracidial cells was found (H&E, ×200). (c) Shows pretreatment of cinnamoylamide to *S. malayensis* infected mice with preserved hepatic lobular architecture. The hepatocytes are within normal limit and preserve its plate pattern. Liver almost returns to the normal pattern (H&E, ×200). (d) Shows pretreatment of *A. gehrtii* leaves extract to *S. malayensis* infected mice with large reserved hepatic lobular architecture and the liver almost returns to the normal pattern (H&E, ×200).

of the control liver showed normal hepatocytes, vascular sinusoids, and centrolobular vein (Figure 3A). *S. malayensis* infection caused liver granuloma with ova embedded in dense fibrous tissue. The thin-walled, non-striated helminth ova are not operculated and contain nonvital miracidial cells (Figure 3B). Examination of liver sections of rats pretreated with *A. gehrtii* leaves extract or cinnamoylamide prior to *S. malayensis* infection showed preserved hepatic lobular architecture. The hepatocytes were within normal limit and preserved its plate pattern. Liver almost returned to the normal pattern (Figure 3C & 3D).

## 4. Discussion

In Malaysia, *S. malayensis*, in addition to *Schnistosoma spindale*, *Schnistosoma nasale*, *Schnistosoma incognitum*, *Trichobilhazia brevis*, and *Pseudobilharziella lonchurae*, is known to occur in wildlife[29]. The *Robertsiella* snails are the intermediate host of *S. malayensis* and have been found almost exclusively in small rivers[30]. Several attempts to recover eggs from feces from the Orang Asli population in peninsular Malaysia[31] were unsuccessful, however, which was attributed to the zoonotic nature of *S. malayensis* and thus missing adaptation to the human host. The rodent (jungle rat) feces could have contaminated the water with the eggs of *S. malayensis*.

The mechanism of *A. gehrtii* protection in *S. malayensis* infection was dependant on the antioxidant activity of the plant amide to reduce the oxidative stress produced by *S. malayensis* infection, where amides at lower concentrations have the ability to reduce schistosome pairing and motility. This effect was due to the functional group of the amine compounds which influenced the schistosomicidal activity[32].

The aim of the present study was to evaluate the protective role of of *A. gehrtii* leaves extract in liver toxicity and apoptosis occurred in *S. malayensis* infection in male albino mice.

The clinical and diagnostic values associated with the changes in blood enzyme concentrations such as AST, ALT, ALP, and serum bilirubin have long been recognized[33,34]. Increased levels of these diagnostic markers of hepatic function in *S. malayensis* infection in male mice are implicative of the degree of hepatocellular dysfunction caused by *S. malayensis* infection. Comparatively lower levels of these parameters in the pretreatment with *A. gehrtii* leaves extract or cinnamoylamide groups to *S. malayensis* mice show the ability of the phyto-constituents to protect the liver against harmful effects of *S. malayensis*.

Albumin is the most abundant circulatory protein and its synthesis is a typical function of normal liver cells. Low levels of albumin have been reported in the serum of patients and animals with hepatocellular cancer[35]. The fall in the serum albumin levels could probably contribute to the low total protein levels observed in *S. malayensis* infected mice. Considerably higher albumin and total protein levels were seen in *A. gehrtii* leaves extract or cinnamoylamide treated groups as compared to *S. malayensis* group, indicating that one of the mechanisms by which the phytoconstituents exhibit their protective effects during cancer is by enhancing the levels of albumin and thereby total protein levels. Thus, based on our preliminary biochemical findings, we suggest possible preventive effects of *A. gehrtii* leaves extract and 0*S. malayensis* infection.

*S. malayensis* increased significantly serum cholesterol, LDL, and triglycerides, while *A. gehrtii* leaves extract or cinnamoylamide pretreatment directed serum cholesterol, LDL, and triglycerides to the normal levels. This effect may be related to amides-containing plant which protects LDLs from oxidation. Lipids targeted for cellular metabolism are mobilized from the intestine as: (Ⅰ) triglycerides-rich chylomicrons; (Ⅱ) triglycerides-rich very-low-density lipoproteins, which are subsequently converted to cholesterol-rich LDL; and (Ⅲ) as cholesterol-phospholipid-rich HDL, which removes cholesterol from peripheral cells and transports it to the liver[36]. Such findings are in agreement with that of Rashed *et al*[37], Xu *et al*[38], and Park

*et al*[39] who found that the amides potently inhibited rabbit liver acyl-CoA: cholesterol O-acyltransferase, and also decreased significantly the plasma cholesterol (42%-68%) in an acute cholesterol-fed rat model.

*S. malayensis* decreased significantly blood GPx and SOD activities but increased serum MDA and plasma NO. On the contrary, *A. gehrtii* leaves extract or cinnamoylamide pretreatment increased blood GPx and SOD activities but decreased serum MDA and plasma NO. This observation could be related to the antioxidant effect of amides-containing plant. Such findings are accepted with that of Marinova *et al*[40] and Storozhok *et al*[41] who found thatcinnamoyl- and hydroxycinnamoyl amides of biogenic amines exhibited an excellent antioxidant activity, higher than or comparable with that of caffeic acid.

In the current study, anti-apoptotic activity of *A. gehrtii* leaves extract or cinnamoylamide was also recorded. P53 and bcl-2 are closely related to the majority of human toxicity and cancer[42]. Apoptotic marker p53 is a critical regulator of apoptosis in many cells. It stimulates a wide network of signals that act through either extrinsic or intrinsic pathways of apoptosis[43] by activating the transcription of downstream genes such as p21 and Bax to induce apoptotic process which inhibit the growth of cells with damaged DNA. On the other hand, bcl-2 has been reported to function primarily by blocking the apoptosis pathway[44]. Bcl-2 gene product is a negative regulator of apoptosis, which forms a heterodimer complex with Bax and neutralizes the effect of pro-apoptosis[45]. Our results indicated that *S. malayensis* decreased p53 expression but increased bcl-2 expression. On the other side, the treatment with *A. gehrtii* leaves extract or cinnamoylamide to *S. malayensis* infected mice increased p53 expression but decreased bcl-2 expression. These results are in agreement with that of Mulware[46] who reported that parasite or environmental toxicant cause induction of oxidative-induced DNA damage by ROS may lead to isolated base lesions or single-strand breaks, complex lesions like double-strand breaks, and some oxidative generated clustered DNA lesions which are linked to cell apoptosis and mutagenesis. On the other side, the *A. gehrtii* leaves extract or cinnamoylamide has anti-apoptotic activity, where the plant leaves extract, an inhibitor of p53, abrogated glucolipotoxicity-induced ROS generation and p53 expression[47].

Histopathological studies of liver tissues of normal, *S. malayensis* infected group and *A. gehrtii* leaves extract or cinnamoylamide + *S. malayensis*-infected groups indicate that either plant extract or alkamide has cytoprotective properties.

Data from the present study highlights *S. malayensis* fatal effects on oxidative stress and apoptosis in the liver of male albino mice. Pretreatment of *A. gehrtii* showed significant reduction in liver oxidative stress and apoptosis in *S. malayensis* evidenced in histological examinations, and this effect was associated with cinnamoylamide ingredient of the plant leaves. However, further clinical studies are warranted to establish its effectiveness and it will be interesting to see whether *A. gehrtii* reverses existing *S. malayensis* liver toxicity and apoptosis, like in real case scenarios.

## Conflict of interest statement

The authors declare that there are no conflicts of interests.

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