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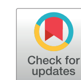
Efficacy of *Ozoroa pulcherrima* Schweinf methanolic extract against *Schistosoma mansoni*-induced liver injury in mice



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A B S T R A C T

The roots of *Ozoroa pulcherrima* Schweinf are used in traditional medicine to treat intestinal helminthiasis. The aim of this study was to assess the effect of *Ozoroa pulcherrima* roots methanolic extract (OPME) on liver injury induced by *Schistosoma mansoni* in mice. A preliminary phytochemical study of OPME was conducted. OPME was given daily and orally to *S. mansoni*-infected mice at 100, 200 or 400 mg/kg for 28 days, starting from the 36th day post-infection. Praziquantel was used as reference drug. Non-infected and infected-untreated mice served as controls. Worm burden and egg output, transaminases, total bilirubin, alkaline phosphatase and total protein; as well as malondialdehyde, catalase and reduced glutathione were evaluated. In OPME, total phenolic was 79.61 ± 0.25 mg gallic acid equivalent/g, while total flavonoid was 7.98 ± 0.04 mg rutin equivalent/g. Treatment of *S. mansoni*-infected mice with OPME produced significant reduction of worm burden and ova count in the faeces, liver and intestine. Significant reduction of alanine aminotransferase activity ($p < 0.001$) as well as significant increase of total protein content ($p < 0.001$) was recorded after OPME treatment at all doses. Total bilirubin level was also reduced ($p < 0.01$). Administration of OPME at all doses corrected the high malondialdehyde level ($p < 0.001$) induced by the infection. At 200 mg/kg, catalase activity and reduced glutathione concentration were significantly increased ($p < 0.001$). OPME at 200 mg/kg showed moderate schistosomicidal effect, but was effective as the standard drug praziquantel in restoring the liver function after *S. mansoni* infection.

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1. Introduction

Schistosomiasis is a chronic parasitic disease caused by a trematode blood fluke of the genus *Schistosoma*. It's one of the most prevalent parasitic diseases in tropics and subtropics and continues to be a significant cause of morbidity and mortality.[1] It is estimated that more than 249 million people are infected and approximately 800 million, mostly children, are at risk in 78 endemic countries.[2] *Schistosoma* infection is one of the most important causes of non-cirrhotic portal hypertension in Latin America, Africa and Asia. *Schistosoma mansoni*-induced liver injury results from a granulomatous inflammatory reaction around trapped *Schistosoma* eggs in

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the presinusoidal periportal spaces. In early phases of the infection, a predominantly hypercellular nonfibrotic granuloma response produces liver dysfunction. Enlargement of the liver is clinically detectable. Chronicity is characterized by liver fibrosis and portal hypertension marked by splenomegaly.[3] In the liver, high rate of oxidative process occurs, precisely the formation of hepatic malondialdehyde due to the peroxidative damage of the liver microsomal membrane lipids. Moreover, impairment of the endogenous antioxidant defense characterizes schistosomiasis.[4,5]

Two decades of ambitious efforts have been devoted to develop an effective vaccine against schistosomiasis without resounding success. For this reason, praziquantel (PZQ) remains the mainstay of the disease control.[6] PZQ has high cure and egg reduction rates with only mild side effects, but suffers from two serious drawbacks. Firstly, it is active only at the adult stage of the worm which means that it exerts its action after sexual maturation and oviposition. Secondly, a series of laboratory studies and clinical trials have raised concerns about the possible development of resistance to PZQ.[7] This urges the need to develop complementary and/or alternative new anthelminthic agents that are both effective and have minimal side effects.[8]

The value of many plant species that have been used in traditional medicine to treat both veterinary and human helminths is increasingly being recognized.[9] Among them, *Nigella sativa, Clerodendrum umbellatum* and *Sida pilosa* have shown good activity against *S. mansoni* infection.[10–12] *Ozoroa pulcherrima* Schweinf (syn.: *Heeria pulcherrrima* Schweinf), family Anacardiaceae, is a small shrub of about 1 m high. The leaves upper surface is puberulous while the underneath is covered with soft. Flowers are small and creamy white and mature fruits are black and stony. It is a sudano-zambezian species, widespread from Guinea to Cameroon, in Central African Republic, in Ethiopia and in Sudan.[13] In Benin, the stem and leaves are used to treat dystocia, hyperthermia and conjunctivitis,[14] while in Cameroon, the root is used to treat dysmenorrhea and intestinal helminthiasis.[13] Three alkylanacardic acids: ozocardic A, ozorcardic A and ozorcardic B have recently been isolated from *O. pulcherrima* roots methanol-dichloromethane extract.[15,16] At the present stage of our literature survey, only one pharmacological study on *O. pulcherrima* has been reported. Essential oils of its aerial parts have been proved to possess antiproliferative activity on breast cancer cells.[17] However, *Ozoroa insignis* which belongs to the same genera, disclosed several activities including antischistosomal, antitumor and antimicrobial.[18–20] The present study was undertaken to assess the effect of the methanolic extract of *O. pulcherrima* roots on *S. mansoni*-induced liver injury in mice.

2. Materials and methods

2.1. Chemicals, reagents and instruments

Praziquantel was supplied by Merck S.A., Mexico, Mexico. Total bilirubin and alkaline phosphatase kits (Inmesco kits) were purchased from Inmesco, Wied, Germany. Transaminases kits were procured from Quibasa Quimica Basica Ldta, Belo Horizonte, Brasil. The following were products of Sigma-Aldrich, St Louis, USA: Ellman's reagent, serum albumin bovine (SAB), trichloroacetic acid (TCA), thiobarbituric acid (TBA), chemicals used for the preparation of the Biuret reagent and buffers. Methanol and others chemicals were of analytical grade. For parasitological study, we used a stereo-microscope (Zeiss, Hamburg, Germany) and a light microscope (Leica, Wetzlar, Germany). For biochemical analyses, we used the Genesys 20 spectrophotometer (Sigma-Aldrich, St Louis, USA).

2.2. Collection of plant material and extraction

Roots of *O. pulcherrima* were harvested in july 2012 in the locality of Wakwa near Ngaoundéré in the Adamawa region of Cameroon. Botanical identification of a plant sample was performed at the "National Herbarium", Yaoundé, Cameroon and a voucher specimen no 13667/SRF/Cam was deposited. The roots were thoroughly washed with water and cut into pieces. They were dried at room temperature and powdered. The powder (4130 g) was submitted to maceration in methanol during 48 h. The macerate was filtered under reduced pressure in a rotary evaporator (BÜCHI B-480) and dried in an oven at 50 °C. Then, we obtained 138.12 g of *O. pulcherrima* roots methanolic extract (OPME).

2.3. Phytochemical screening

The methanolic extract of *O. pulcherrima* roots was subjected to qualitative chemical tests in a bid to identify phytochemical constituents in the fraction. The screening of alkaloids, anthraquinones and cardiac glycosides was performed using the Mayer's test, the Bornträger's test and the Keller-Killiani's test respectively. The following tests were used: Ferric chloride test ($FeCl_3$), for the identification of phenols and tannins, Fehling's test for reducing sugars, foam test for saponins and Liebermann-Burchard's test for steroids and triterpenes. The presence of flavonoids was revealed by the ammonia test, that of lipids by the grease spot test and terpenoids, by the Salkowski's test.[21]

2.4. Determination of the total phenolic content and flavonoid content in the extract

Folin-Ciocalteu's method described by Mansouri *et al.* was used to establish the total phenolic content in OPME.[22] Total phenolic content was calculated from the calibration curve of gallic acid serial dilutions and expressed as mg of gallic acid equivalents (GAE) per g of dried extract. Spectrophotometric method described by Pal *et al.* was used to calculate the total flavonoid content in OPME.[23] Total flavonoid content was estimated by using a calibration curve of rutin and expressed as mg rutin equivalents per g of dried extract.

2.5. Animals and infection

Eight-week-old Balb/c mice, weighing 20–25 g bred at the "Centre for Schistosomiasis and Parasitology" of Yaoundé – Cameroon, were used for the study. All animals were housed in polypropylene cages with free access to food and water and maintained under natural 12 h light/12 h dark cycles and temperature between 22 and 25 °C. Animals were individually infected with 50 cercariae of *S. mansoni* (cameroonian strain) by the tail and legs immersion technique as described by Oliver and Stirewalt.[24] Cercariae were released from *Biomphalaria pfeifferi* snails, collected from the river "Afeme" (Yaoundé, Cameroon) and maintained in the laboratory under standardized conditions. All procedures in this study followed the principles of laboratory animal use and care of the "European Community" guidelines (EEC Directive 2010/63/EEC) and were approved by the "Animal Ethical Committee" of the Laboratory of Animal Physiology of the Faculty of Sciences, University of Yaoundé I – Cameroon.

2.6. Experimental design

S. mansoni-infected animals were divided into five (5) groups. Infected-untreated group, named Group IC ($n = 6$) was composed of mice receiving 1% dimethyl sulfoxide (DMSO) (vehicle); group

PZQ (n = 6) was composed of mice receiving praziquantel at the dose of 100 mg/kg for 5 consecutive days followed by distilled water till the end of the experiment. In groups OPME 100 (n = 5), OPME 200 (n = 6) and OPME 400 (n = 6), mice were treated with *O. pulcherrima* methanolic extract at 100, 200 and 400 mg/kg respectively, for 28 consecutive days. The period of treatment and the choice of doses were done according to the prescription of traditional healers and on the basis of our previous findings.[11–13] All treatments started on the 36th day post-infection. The extract or praziquantel dissolved in 1% DMSO was given daily and *per os*. In addition, a group of uninfected mice named group HC (n = 6) was used and received vehicle only (1% DMSO). All animals were sacrificed on the 65th day post-infection by cervical dislocation.

2.7. Measuring body and organs weights

During the course of the experiment, each mice was weighed once a week in order to assess body weight variation between pre-infection and post-infection (65th day). The percentage of body weight gain was calculated as follows:

$$P = [(W_e - W_i) / W_e] \times 100$$

where P = percentage of weight gain; W_e = body weight at post-infection; W_i = body weight at pre-infection.

After sacrifice, liver and spleen were removed from each mouse, weighed and their relative weights (g of organ/100 g of body weight) was calculated.

2.8. Worm burden

On the 65th day post-infection, adult *S. mansoni* worms were recovered by perfusion from the porto-mesenteric system and the liver, as described by Duvall and Dewitt.[25] Recovered worms from each infected mouse were counted under a stereo-microscope. The percentage of reduction of worm number was calculated as follows [26]:

$$P = [(C - V) / C] \times 100$$

where P = percentage of reduction; C = mean number of worms recovered from infected-untreated mice; V = mean number of worms recovered from infected-treated mice.

2.9. Egg count in the faeces, liver and intestine

Faeces were collected from each infected mice the day before the sacrifice and weighed. After homogenization in 10 % buffered formaldehyde, the mixture was stored at 4 °C. Two aliquots of 100 μL each were counted on light microscope. After the sacrifice, the liver and the intestine were removed and weighed. They were digested separately in 4% KOH solution at 37 °C for 6 h until no sign of intact tissue was present. Tissue suspensions were then centrifuged at 1500 rpm for 5 min and supernatants removed.[27] After three cycles of washing and centrifugation, the number of eggs was determined in two aliquots of 100 μL each using a light microscope. Results were expressed in terms of mean number of eggs per gram of faeces or per gram of tissue for the liver and the intestine.

2.10. Evaluation of some liver function biomarkers

Some parameters of the liver function were measured in the plasma after the sacrifice. Blood from each mouse was then collected from the retro-orbital venous plexus in EDTA tube and centrifuged at 3500 rpm for 15 min. Plasma obtained was stored at −70 °C for biochemical analysis. Total protein level was determined using the method of Biuret.[28] The amount of protein was calculated from a standard curve using serial concentrations of bovine serum albumin. Activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were assessed using Bioclin kits according to the method of Reitman and Frankel.[29] Alkaline phosphatase was estimated according to Tietz *et al.* by using Inmesco kit.[30] The decrease in absorbance was measured at 410 nm at 1 min intervals for 3 min and activities expressed as U/l. Total bilirubin was assayed according to the protocol described in the Inmesco kit. The red color developed after the reaction of bilirubin with sulfanilic acid was read at 546 nm against a blank.

2.11. Evaluation of some oxidative stress biomarkers in the liver

The liver (0.4 g) was homogenized in 2 ml of a Tris-HCl 50 mmol buffer, pH 7.4. The homogenate (20% w/v) was centrifuged at 3000 rpm for 25 min at 4 °C. The supernatant was collected and stored at -70 °C until assayed. Lipid peroxides were measured as thiobarbituric acid reactive substances. The product of the reaction between malondialdehyde and TBA was estimated as described by Wilbur *et al.*[31] The concentration of MDA was calculated using a molar extinction coefficient of 1.56×10^{-5} $mmol^{-1}cm^{-1}$ and results expressed as nmol/g of liver. Catalase activity was assayed following the protocol described by Sinha.[32] Catalase activity was calculated from the standard curve and expressed as mol/min/g of liver. Reduced glutathione (GSH) was determined using the Ellman's reagent (5 mg of 2, 2-dithio-5, 5-nitrobenzoic acid + 250 ml phosphate buffer), as described by Ellman.[33] The concentration of GSH was calculated using a molar extinction coefficient of 13600 $mol^{-1}cm^{-1}$and results expressed as mmol/g of liver.

2.12. Statistical analysis

Data are expressed as mean ± SEM. Data were analyzed using GraphPad Prism version 4.00 for Windows, by one-way analysis of variance (ANOVA) and differences between groups were assessed using the Newman-Keuls multiple comparison post-test. Differences were considered significant from $P < 0.05$.

3. Results

3.1. Phytochemical constituents of O. pulcherrima roots methanolic extract

The qualitative phytochemical analysis of *O. pulcherrima* roots methanolic extract revealed the presence of anthraquinones, terpenoids, flavonoids, saponins, tannins, phenols, cardiac glycosides, alkaloids, triterpenes and lipids.

In *O. pulcherrima* roots methanolic extract, the total phenolic content was found to be 79.61 ± 0.25 mg/g of dried extract, calculated as gallic acid equivalent ($r^2 = 0.9994$) and the total flavonoid content was 7.98 ± 0.04 mg/g of dried extract, calculated as rutin equivalent ($r^2 = 0.9959$).

3.2. Effect of O. pulcherrima methanolic extract on the body, liver and spleen weights

As shown in Table 1, mice of all groups gained weight, although the weight gain of infected-untreated mice (IC) and infected-treated mice (PZQ, OPME 100, OPME 200, OPME 400) was significantly lower than the one of healthy mice (HC). However, the body weight gain of animals treated with praziquantel or *O. pulcherrima* methanolic extract at 100 or 400 mg/kg showed a significant increase ($p < 0.05$) as compared to infected-untreated mice (IC). No

Table 1
Effect of *Ozoroa pulcherrima* methanolic extract on the body weight and organs weights of *Schistosoma mansoni*-infected mice.

Groups	Body weight (g)		Body weight gain (%)	Liver weight (g/100 body weight)	Spleen weight (g/100 body weight)
	Pre-infection	Post-infection			
HC	18.38 ± 0.56	26.08 ± 0.80	29.19 ± 3.04 (21.38–37.00)	6.69 ± 0.62	0.58 ± 0.04
IC	23.93 ± 1.10	25.57 ± 0.66	6.36 ± 2.91 (-1.13–13.86)***	8.50 ± 0.69*	1.36 ± 0.14***
PZQ	21.22 ± 1.04	26.00 ± 0.98	18.41 ± 1.84 (13.68–23.14)**,#	6.57 ± 0.46##	0.43 ± 0.08###
OPME 100	19.78 ± 0.85	23.99 ± 1.22	17.25 ± 2.57 (10.12–24.38)*,#	7.73 ± 0.39	0.76 ± 0.07###
OPME 200	22.81 ± 0.85	26.42 ± 1.27	13.31 ± 2.24 (7.56–19.06)***	7.02 ± 0.34	0.64 ± 0.10###
OPME 400	22.00 ± 0.80	26.76 ± 1.03	17.57 ± 2.34 (11.55–23.59)**,#	8.35 ± 0.65	0.86 ± 0.12##, £

Data are expressed as mean ± SEM (n = 5 for group OPME 100 and n = 6 for others groups). HC: healthy animals; IC: infected-untreated animals; PZQ: infected animals treated with praziquantel; OPME 100, OPME 200 and OPME 400: infected animals treated with the methanolic extract of *O. pulcherrima* roots at 100, 200 and 400 mg/kg respectively. ANOVA followed by Newman-Keuls multiple comparison test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$: values are significantly different from the healthy animals (group HC). #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$: values are significantly different from the infected-untreated animals (group IC). £$p < 0.05$: values are significantly different from the infected animals treated with praziquantel (group PZQ). Values in brackets represent the 95 % confidence intervals.

statistical difference was recorded between *O. pulcherrima*-treated groups and PZQ-treated group (Table 1).

S. mansoni infection induced a significant increase of the liver weight ($p < 0.05$) and spleen weight: ($p < 0.001$) by 27.06% and 134.48% respectively when compared to healthy mice (HC). Comparatively to the liver weight of infected-untreated mice, the one of mice treated with praziquantel or OPME at 100 or 200 mg/kg was reduced. This reduction was statistically significant only in group PZQ ($p < 0.01$). PZQ or OPME treatment also induced significant decrease of the spleen weight ($p < 0.001$) by 68.38% (PZQ), 44.12% (OPME 100), 52.94% (OPME 200) and 36.76% (OPME 400) as compared to infected-untreated mice. The spleen weight of mice treated with OPME at 100 or 200 mg/kg was comparable to that of PZQ-treated mice (Table 1).

3.3. Effect of O. pulcherrima methanolic extract on parasitological parameters

The worm burden of infected-untreated mice was 16.67 ± 1.99 worms. Treating the infected mice with OPME at 100, 200 or 400 mg/kg resulted in a significant decrease ($p < 0.001$) of worm count by 53.21, 74.02 or 67.01% respectively, as compared to infected-untreated mice. Praziquantel treatment induced a high reduction of worm burden by 86.98% ($p < 0.001$). The worm burden of mice treated with OPME at 200 or 400 mg/kg was comparable to that of PZQ-treated mice (Table 2).

Compared to infected-untreated mice, ova count in the faeces was significantly reduced by 92.45, 93.30 and 91.70% after administering the extract to infected mice at 100, 200 or 400 mg/kg respectively. Moreover, after treatment with OPME, a significant reduction of ova count in the liver by 75% ($p < 0.05$) was recorded only at the dose of 200 mg/kg. In the intestine, the ova burden decreased significantly: 69.30, 89.28 or 81.43% at 100, 200 or 400 mg/kg of OPME respectively. Treatment with praziquantel resulted in significant reduction of ova count by 100% in the faeces, 99.29% in liver and 99.91% in the intestine. Despite its significant reduction, the intestine ova burden of mice treated with OPME at 100 mg/kg was higher than in PZQ-treated mice. Between praziquantel and OPME at 200 mg/kg, there was no statistical significant difference in their capacity to reduce ova burden in the faeces, the liver and the intestine (Table 2).

3.4. Effect of O. pulcherrima methanolic extract on some parameters of the liver function

The present study showed that *S. mansoni* infection induced significant increase of transaminases activities (ALT and AST) in the plasma. Oral administration of praziquantel or OPME at the doses of 100, 200 or 400 mg/kg to infected mice resulted in significant decrease of ALT activity by 50.50%, 36.90%, 50.77% or 28.19% respectively, as compared to infected-untreated mice. OPME at 100 and 200 mg/kg was as effective as praziquantel. A significant reduction of AST activity ($p < 0.05$) was recorded only in PZQ-treated mice (Table 3).

The total bilirubin concentration which was increased by the infection ($p < 0.001$), showed a significant reduction by 36.69%, 52.48% or 24.92% after treatment of infected mice with PZQ or the extract at 200 or 400 mg/kg respectively. The efficacy of OPME at these two doses was comparable to that of praziquantel in reducing total bilirubin level (Table 3).

As shown in Table 3, *S. mansoni* infection resulted in significant reduction of total proteins level and alkaline phosphatase (ALP) activity in the plasma. Compared to infected-untreated animals, total protein level significantly increased ($p < 0.001$) by 58.28%, 57.14%, 45.71% or 65.14% after treatment with PZQ or OPME at 100, 200 or 400 mg/kg respectively. Used at different doses, OPME was as effective as praziquantel, since no statistical significant difference was recorded between OPME-treated groups and PZQ-treated

Table 2
Effect of *Ozoroa pulcherrima* methanolic extract on the worm burden and ova count in the feces, the liver and the intestine of *Schistosoma mansoni*-infected mice.

Groups	Worm burden	Ova count (number of ova/g of organ)		
		Feces	Liver	Intestine
IC	16.67 ± 1.99	2639.50 ± 602.51	3534.65 ± 1165.61	19549.60 ± 2700.30
PZQ	2.17 ± 0.70###	00.00 ± 00.00###	25.13 ± 18.39##	17.86 ± 11.40###
OPME 100	7.80 ± 1.43###, £	199.16 ± 62.88###	2614.45 ± 543.70	6001.81 ± 1147.89###, £
OPME 200	4.33 ± 1.05###	176.88 ± 29.81###	883.58 ± 260.44#	2095.17 ± 469.72###
OPME 400	5.50 ± 1.36###	218.98 ± 68.04###	2205.99 ± 601.62	3629.90 ± 646.36###

Data are expressed as mean ± SEM (n = 5 for group OPME 100 and n = 6 for others groups). IC: infected-untreated animals; PZQ: infected animals treated with praziquantel; OPME 100, OPME 200 and OPME 400: infected animals treated with the methanolic extract of *O. pulcherrima* roots at 100, 200 and 400 mg/kg respectively. ANOVA followed by Newman-Keuls multiple comparison test. #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$: values are significantly different from the infected-untreated animals (group IC). £$p < 0.05$: values are significantly different from the infected animals treated with praziquantel (group PZQ).

Table 3
Effect of *Ozoroa pulcherrima* methanolic extract on some parameters of the liver function of *Schistosoma mansoni*-infected mice.

Groups	Total proteins (mg/mL)	AST (U/mL)	ALT (U/mL)	ALP (U/L)	Total bilirubin (μmol/L)
HC	2.78 ± 0.07	159.27 ± 6.48	345.57 ± 37.48	41.36 ± 6.72	8.11 ± 0.39
IC	1.75 ± 0.08***	303.09 ± 64.92*	791.29 ± 32.20***	8.96 ± 2.58**	24.20 ± 1.68***
PZQ	2.77 ± 0.09###	168.13 ± 13.41#	391.66 ± 31.85###	28.95 ± 9.14	15.32 ± 1.76###
OPME 100	2.75 ± 0.19###	234.23 ± 6.93	499.32 ± 54.40###	7.44 ± 2.25	22.21 ± 1.03
OPME 200	2.55 ± 0.19###	195.26 ± 15.37	389.51 ± 42.33###	20.91 ± 6.65	11.50 ± 1.41###
OPME 400	2.89 ± 0.08###	231.93 ± 18.03	568.18 ± 37.66###, ££	15.62 ± 2.82	18.17 ± 1.20##

Data are expressed as mean ± SEM (n = 5 for group OPME 100 and n = 6 for others groups). AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase; HC: healthy animals; IC: infected-untreated animals; PZQ: infected animals treated with praziquantel; OPME 100, OPME 200 and OPME 400: infected animals treated with the methanolic extract of *O. pulcherrima* roots at 100, 200 and 400 mg/kg respectively. ANOVA followed by Newman-Keuls multiple comparison test. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$: values are significantly different from the healthy animals (group HC). #$p < 0.05$; ##$p < 0.01$, ###$p < 0.001$: values are significantly different from the infected-untreated animals (group IC). ££$p < 0.01$: values are significantly different from the infected animals treated with praziquantel (group PZQ).

group. The activity of ALP did not varied significantly after administration of OPME (Table 3).

3.5. Effect of O. pulcherrima methanolic extract on some liver oxidative stress biomarkers

This study showed that *S. mansoni* infection induced significant increase of malondialdehyde concentration in the liver by 332.64% ($p < 0.001$). Treatment with OPME at 100, 200 or 400 mg/kg, showed significant decrease of hepatic malondialdehyde by 74.87%, 68.01% or 66.26% respectively as compared to infected-untreated mice ($p < 0.001$). This result was comparable to that obtained with praziquantel (Fig. 1A).

S. mansoni infection also induced a significant reduction ($p < 0.001$) of catalase activity by 81.38% and reduced glutathione level by 39.37% (Fig. 1B and C). Regarding catalase activity, treatment with praziquantel or OPME at 200 or 400 mg/kg resulted in significant increased by 437.12%, 595.37% or 275.19% respectively as compared to infected-untreated mice. OPME at these doses was effective as praziquantel (Fig. 1B). Reduced glutathione concentration showed significant increase by 43.68% or 60.34% in mice treated with OPME at 100 or 200 mg/kg respectively, as compared to infected-untreated mice. Treatment with praziquantel induced no variation of reduced glutathione concentration and OPME at 200 mg/kg was more effective than praziquantel ($p < 0.01$) (Fig. 1C).

4. Discussion

S. mansoni infection is mostly due to *Schistosoma* eggs trapped in the host tissues where they induce inflammatory and fibrotic lesions. It's one of the most important causes of non-cirrhotic portal hypertension in Latin America, Africa and Asia.[3] Epidemiological studies conducted in *S. mansoni*-endemic regions in Brazil and in Africa have revealed that *S. mansoni* infection is associated with growth retardation and chronic hepatosplenomegaly among children.[34,35] Results from Table 1 revealed that *S. mansoni*-infected mice exhibited significant growth retardation and hepatosplenomegaly. Enlargement of the liver is thought to be due to eggs deposition in the hepatic tissue and enlargement of the spleen is due to passive congestion of blood flow and reticuloendothelial hyperplasia.[35,36] The mechanism behind hepatosplenomegaly is probably related to immune response. In fact, Mwatha *et al.* have demonstrated that childhood hepatosplenomegaly is associated with high levels of Th1 response (TNF and IFNγ).[37] A 3-year mass drug administration with praziquantel has shown to be effective in reducing hepatomegaly and splenomegaly around the Poyang Lake region in China.[38] In this study, as shown on Table 1, administration of praziquantel to *S. mansoni*-infected mice resulted in a significant reduction of hepatomegaly and splenomegaly. Also, OPME treatment improves the growth of infected mice significantly as well as it reduces their spleen weight. The reduction of hepatosplenomegaly could be the consequence of the reduction of the number of eggs trapped in different organs. This study has effectively disclosed the reduction of egg load in the liver and intestine after praziquantel or OPME treatment (Table 2). The reduction of the worm burden as reported in this study will lead to the reduction of the egg load in the faeces, the liver and the intestine. OPME is lethal to *S. mansoni* adult worms as the aqueous extract and the organic fractions of some medicinal plants.[10–12] Chemical compounds with anthelmintic property could be responsible for the antischistosomal activity of OPME. The phytochemical screening of this extract has revealed the presence of alkaloids, flavonoids, tannins and terpenoids which are directly active against helminths, affecting their viability, mobility and fecundity.[39,40] The results of this study are in line with findings reported by Jatsa *et al.* who attributed the antischistosomal activity of *C. umbellatum* and *S. pilosa* to alkaloids and tannins present in those plants extracts.[11,12] The antischistosomal potential of OPME could be attributed to alkaloids, flavonoids, tannins and terpenoids present in the extract.

In *S. mansoni* infection, the granuloma response produces liver dysfunction. The degree of hepatoprotection by OPME was measured by means of estimating biochemical parameters, such as transaminases, total bilirubin, alkaline phosphatase (ALP) and total protein in the plasma. High deposition of schistosome eggs in the liver induces hepatocellular injury, which in turn, leads to the release of transaminases from the injured hepatic cells to the bloodstream. Hence, the increase of plasmatic activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[40,41] The current investigation showed that *S. mansoni* infection induced significant increase of AST and ALT activities in the plasma. There was a considerable rise of total bilirubin concentration while ALP activity and total protein concentration decreased significantly (Table 3). High levels of serum bilirubin generally reflect an imbalance between production and conjugation. During the course of schistosomiasis, worms continuously deteriorate hemoglobin from the host, thus increasing the concentration of bilirubin, a yellow pigment generated by hemoglobin breakdown.[42,43] Moreover, a rise of direct bilirubin is very specific for biliary tract obstruction by schistosomes and their eggs.[44] The reduction of alkaline phosphatase activity is generally related to severe anemia as recorded in patients suffering from schistosomiasis.[42] *S. mansoni* infection is also characterized by a reduction of the activity of enzymes associated with citric acid cycle, fatty acid cycle, urea cycle as well as amino acid metabolism but an increase of the level of proteins associated with stress responses, acute phase reactants and structural components.[45] As shown on Table 3, administration of OPME to *S.mansoni*-infected mice leads to a significant reduction of ALT activity in the plasma. Moderate to low levels of ALT and AST in blood denote a healthy liver or a liver which is recovering from a

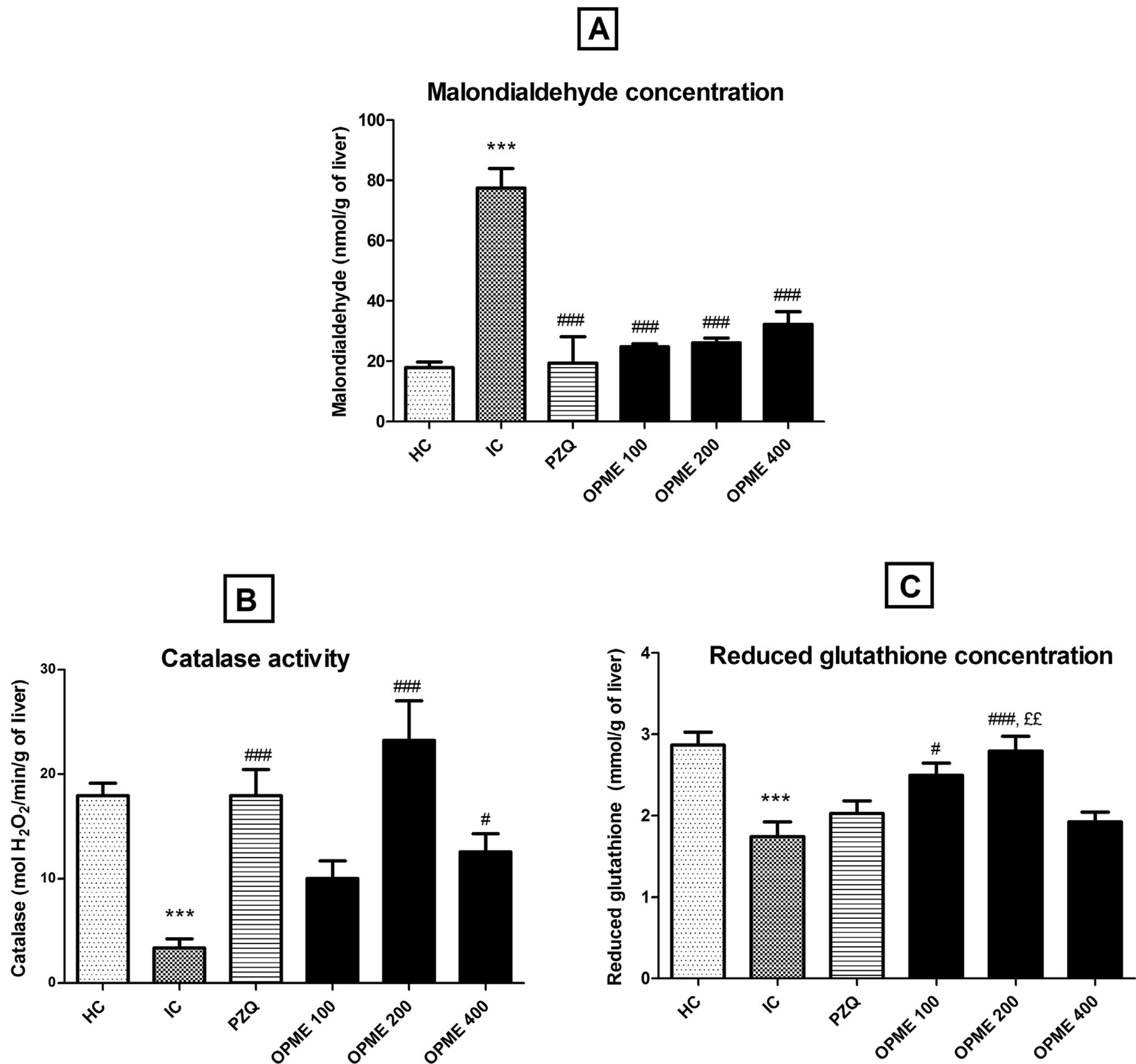

Fig. 1. Data are expressed as mean ± SEM (n=5 for group OPME 100 and n=6 for others groups). HC: healthy animals; IC: infected-untreated animals; PZQ: infected animals treated with praziquantel; OPME 100, OPME 200 and OPME 400: infected animals treated with the methanolic extract of *O. pulcherrima* roots at 100, 200 and 400 mg/kg respectively. ANOVA followed by Newman-Keuls multiple comparison test. $^{***}p < 0.001$: values are significantly different from the healthy animals (group HC). $^{\#}p < 0.05$; $^{\#\#\#}p < 0.001$: values are significantly different from the infected-untreated animals (group IC). $^{££}p < 0.01$: values are significantly different from the infected animals treated with praziquantel (group PZQ).

given disease in most cases.[42] The beneficial effect of OPME in protecting the liver can be attributed to secondary metabolites as anthraquinones, alkaloids, flavonoids, tannins, phenols, terpenoids and saponins present in the extract. These phytochemicals possess anti-inflammatory activity and probably act by reducing the migration of inflammatory cells such as eosinophils, neutrophils, lymphocytes and macrophages around schistosome eggs trapped in the liver.[46–49] Through its antiproliferative activity, cardiac glycosides could also protect the liver from the invasion of inflammatory cells.[50] Antiproliferative property of essential oils rich in hydrogenated monoterpenes from *O. pulcherrima* leaves has been demonstrated by Bogninou-Agbidinoukoun *et al.*[17] Furthermore, the hepatoprotective role of flavonoids, tannins, saponins and terpenoids has been demonstrated in other models of hepatotoxicity. Flavonoids and tannins effectively reduce the hepatic toxicity of alcohol, acetaminophen and hepatocarcinogenic compounds and protect hepatocytes from oxidative stress.[51] Saponins stimulate the protein synthesis which accelerates the regeneration process and the production of liver cells.[52] Terpenoids inhibit the effect of carbon tetrachloride on the liver mitochondrial swelling and consequently prevent the membrane fragility, the enzyme leakage and the hepatocytes degeneration.[53] Hepatocellular regeneration resulting from the anti-inflammatory activity of OPME phytochemicals might explain the restoration of ALT activity, total bilirubin concentration and total protein level after the treatment of *S. mansoni*-infected with OPME.

In *S. mansoni* infection, oxidative stress occurs in the liver at the site of inflammation in the vicinity of *S. mansoni* eggs. Schistosomiasis reduces the level of protective endogenous antioxidants and increases generation of free radicals that leads to a state of oxidative

stress.[4,5,54–56] The overproduction of reactive oxygen species (ROS) induces lipid peroxidation that leads to an increase of malondialdehyde concentration in liver tissue.[46] Our findings presented on Fig. 1 revealed a significant increase of malondialdehyde concentration and a significant decrease of catalase activity and reduced glutathione concentration in *S. mansoni*-infected mice. These results are in agreement with the literature on both human and experimental *S. mansoni* infection.[11,12,54–57] Depletion of catalase activity mostly resulted from its utilization in scavenging reactive oxygen species overload generated during schistosome infection.[46,54,58] El Rigal *et al.* attributed the reduction of reduced glutathione level to the increasing oxidative stress and the cytotoxicity of hydrogen peroxide.[56] OPME treatment of *S. mansoni*-infected animals restored malondialdehyde concentration as well as catalase activity and reduced glutathione level (Fig. 1). This result demonstrated the capacity of the extract to scavenge reactive oxygen species, thus protecting the liver from their deleterious effect. Such antioxidant potential is probably linked to flavonoids, phenols, tannins, saponins and terpenoids present in the extract. In fact, total phenolic and flavonoid contents have been quantified in OPME. Thus, OPME phenolic compounds can donate hydrogen to free radicals and break the chain reaction of lipid oxidation. Flavonoid compounds might limit reactive oxygen species formation and scavenge them, while tannins can stop lipid oxidation. In addition, flavonoid compounds are able to increase antioxidant synthesis by the liver cells.[58] Antiperoxidative and antioxidant properties have been also ascribed to saponins. In normal rats, crude saponins from the fruits of *Solanum anguivi* significantly decreased the malondialdehyde concentration and increased superoxide dismutase and catalase activities.[59] Furthermore, the role of triterpenoids in scavenging free radicals as superoxide anions and hydroxyl radicals has been demonstrated by Gao *et al.*[53] and Wu *et al.*[60] These overall actions would contribute to increase the liver antioxidant capacity and protect it from oxidative stress.

5. Conclusion

The treatment of *S. mansoni* infection with the methanolic extract of *O. pulcherrima* roots at the medium dose of 200 mg/kg showed a reduction of worm and egg burdens as well as an increase of the anti-inflammatory and the antioxidant capacities of the host. These activities were comparable to those disclosed by the standard drug praziquantel. The present work suggested that *O. pulcherrima* roots methanolic extract could be the source of new molecules against hepatic dysfunction in *S. mansoni* infection. Toxicity tests are needed in further investigation to ensure the safety of this extract.

Conflict of interests

The authors declare that there is no conflict of interest regarding the publication of this paper.

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