



## Original article

Ameliorative role of *Ziziphus spina-christi* leaf extracts against hepatic injury induced by *Plasmodium chabaudi* infected erythrocytesTaghreed A. Hafiz<sup>a,\*</sup>, Murad A. Mubarak<sup>a</sup>, Marwa S.M. Diab<sup>b</sup>, Mohamed A. Dkhil<sup>c,d</sup>, Saleh Al-Quraishy<sup>b</sup><sup>a</sup> Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, Saudi Arabia<sup>b</sup> Molecular Drug Evaluation Department, National Organization for Drug Control & Research (NODCAR), Giza 12553, Egypt<sup>c</sup> Department of Zoology, College of Science, King Saud University, Saudi Arabia<sup>d</sup> Department of Zoology and Entomology, Faculty of Science, Helwan University, Egypt

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## ABSTRACT

One of the most common deadliest parasitic diseases is Malaria. The biology and the pathogenesis of this fascinating parasite are not yet fully understood which make discovering effective alternative drugs a challenging task. Moreover, the emergence of resistant strains added an additional burden in the journey of malaria elimination. Traditional medicine used to be an alternative therapy choice owing to the presence of potent natural products. *Ziziphus spina-christi* (L.) considered being one of the common potent natural plant in gulf region and other nations. Therefore, this study designed to evaluate the ameliorative role of *Z. spina-christi* leaf extracts (ZSCL) against *Plasmodium chabaudi*-induced hepatic injury. The study involved three groups were as follows; a vehicle control group, infected with 10<sup>6</sup>*P. chabaudi*-parasitized erythrocytes group and ZSCL treated-infected mice with 10<sup>6</sup>*P. chabaudi*-parasitized erythrocytes group. The results showed a remarkable reduction of parasitemia level and notable reverse of the anemic picture among ZSCL treated-infected mice. The effects of ZSCL on the liver functions enzymes and on the histopathological pictures of liver were significant. It could be concluded that *Z. spina-christi* leaf extracts have a protective role against *Plasmodium* infection that also marked through significant restoration of hepatic oxidative markers.

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## 1. Introduction

Malaria is a life-threatening blood disease that still causing high mortality rate regardless to all the efforts that have been made in preventing the disease. In fact, three main integrated actions are considered the power in preventing malaria which is vector control, effective vaccination and potent antimalarial drug. Application of Vector control targets reduction of malaria transmission (Benelli, 2015) whereas malaria vaccine, currently in clinical phase III trials, aims toward malaria elimination (Mian-McCarthy et al., 2012).

\* Corresponding author at: Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, P.O. Box: 10219, Riyadh 11433, Saudi Arabia.

E-mail address: [thafiz@ksu.edu.sa](mailto:thafiz@ksu.edu.sa) (T.A. Hafiz).

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For antimalarial drugs, emerging resistance against effective drugs like artemisinin-based combination therapies as well as the previous first-line therapies has complicated malaria treatment (Kumar et al., 2015). Therefore as continuing to all the efforts in fighting malaria, scientist explored different potential therapy in the folkloric medicine. *Ziziphus spina-christi* (L.) Willd (ZSC), common names Sidr in Arabic and Christ's thorn or Jerusalem-thorn in English, is a member of Rhamnaceae family. The genus *Ziziphus* has medicinal importance and has been used to maintain a healthy life style by the local Arab people. *Z. spina-christi* has been reported to have antimicrobial activity against pathogens that are normally quite resistant to modern medications (Nazif, 2002). Recent studies showed a significant effect of *Z. spina-christi* on *Plasmodium berghei* parasite induced hepatic and spleen tissue damage (Hafiz and Mubarak, 2016). Therefore, examining the liver during malaria is informative as liver pathology usually varies from slight discrepancies in liver function tests to severe liver failure (Anand and Puri, 2005, Whitten et al., 2011). Indeed, the fascinating life cycle of *Plasmodium* parasite explains the importance of liver where the clinically silent pre-erythrocytic stages of the parasite took place (Cowman and Crabb, 2006).

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Regarding the study model, previous studies have used *Plasmodium chabaudi* rodent malaria parasite as an appropriate model to study the role of liver during malaria infection since it shared many of the pathological and immunological features with the human malaria parasite, *P. falciparum* (Hall et al., 2005). Therefore, the current work proposed to study the Ameliorative role of *Z. spina-christi* leaf extract against hepatic injury induced by *P. chabaudi*-infected erythrocytes.

## 2. Materials and methods

### 2.1. Leaf extracts preparation

*Ziziphus spina-christi* leaf extracts (ZSCLE) was prepared with minor modifications according to Hafiz and Mubarak (2016). A defined amount of ZSCLE (100 g) in air dried powder was extracted using 70% methanol at 27 °C. Then it was reserved at 4 °C for 24 h. Reduced pressure (bath temperature 50 °C) was used to concentrate the extracts before being dried in a vacuum evaporator. For experimental use, distilled water was used to dissolve the residue.

### 2.2. Mice and *P. chabaudi* infection

Using the animal house facility at King Faisal Hospital, Riyadh, adult C57BL/6 male mice were obtained. Animals were maintained in a specific pathogen-free condition upon strict agreement with the institutional and national official guideline at the department of Zoology animal housing facilities. *P. chabaudi* was passaged in Swiss albino mice and once parasitemia reached 30%, infecting mice with parasitized blood was conducted. An intraperitoneal injection of  $1 \times 10^6$  *P. chabaudi*-infected erythrocytes was inoculated to the designated groups of mice. Giemsa stain was used for blood smears staining and parasitemia were calculated. To estimate the cell number per ml, the Neubauer chamber was used.

### 2.3. Experimental design

Three groups were as follows; a vehicle control group, infected with  $10^6$  *P. chabaudi*-parasitized erythrocytes group and ZSCLE treated-infected mice with  $10^6$  *P. chabaudi*-parasitized erythrocytes group. The last group was gavage with 100  $\mu$ l of 300 mg/kg ZSCLE for 8 days (Alzahrani et al., 2016). At day 8 post-infection (p.i.), scarification of all mice was performed.

### 2.4. Liver function tests

The blood plasma was separated and kept at  $-20$  °C until use. The plasma was then analyzed using commercial kits according to the instructions of the manufacturer (BioMérieux, Marcy-l'Étoile, France) for alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP).

### 2.5. Histopathology of liver

Liver was aseptically extracted and fixed in neutral buffered formalin. Following fixation, dehydrating, embedding specimens in wax, 5 microns thickness sections were prepared. Haematoxylin and eosin stains were used to stain the sections for histological examinations.

### 2.6. Oxidant/antioxidant status

Phosphate buffer was used for homogenization of known weights of liver tissues. 50 mM Tris-HCl ice-cold medium containing 300 mM sucrose at pH 7.4 was used for homogenization

according to (ADZU et al., 2007). Liver homogenates were used for biochemical studies.

#### 2.6.1. Malondialdehyde (MDA) level

MDA level in liver homogenate of mice was measured according to the method of (Ohkawa et al., 1979) where the formed thiobarbituric acid reactive substances absorbance was determined at 535 nm.

#### 2.6.2. Catalase

The activity of catalase in the liver homogenate was determined following the method of (Aebi, 1984). In this assay, a known quantity of  $H_2O_2$  was added, after which the reaction was stopped with a CAT inhibitor. The remaining  $H_2O_2$  reacts with 3,5 dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone in the presence of horseradish peroxidase, resulting in a chromophore color. The absorbance was measured at 240 nm.

### 2.7. Statistical analysis

A statistical package program (SPSS version 17.0) was used to perform one-way ANOVA and statistical comparisons between the groups with Duncan's test.  $P \leq .05$  was considered as significant for all the statistical analyses.

## 3. Results

Aiming toward evaluating the antiplasmodial and antioxidant role of ZSCLE, parasitemia level, hematological and biochemical liver analysis have been conducted. Firstly, observing the parasitemia level provide an initial indication of the ZSCLE effectiveness against *P. chabaudi*-induced hepatic injury. In comparison with untreated infected group, parasitemia level was reduced significantly in ZSCLE treated infected mice (Fig. 1). This reduction may highlight the possible antimalarial activity of ZSCLE. This effect might be referred to data indicated that the methanolic leaf extract of *Z. spina-christi* contains high content of both of phenolic and flavonoid compounds (Fig. 2).

Expectedly, in accordance with high parasitemia level during *P. chabaudi* infection, anemic signs were observed as haemoglobin level and erythrocytes counts showed a notable reduction in comparison with control group. Significantly, ZSCLE treated infected mice record a restoration of haemoglobin level and erythrocytes counts as well as a reduction in the inflammatory cells count

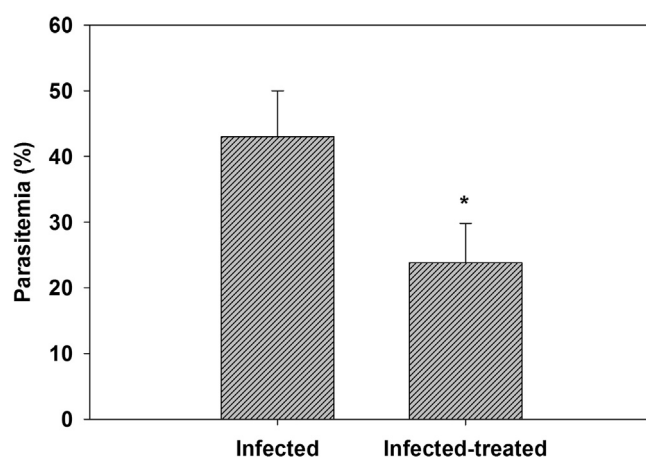
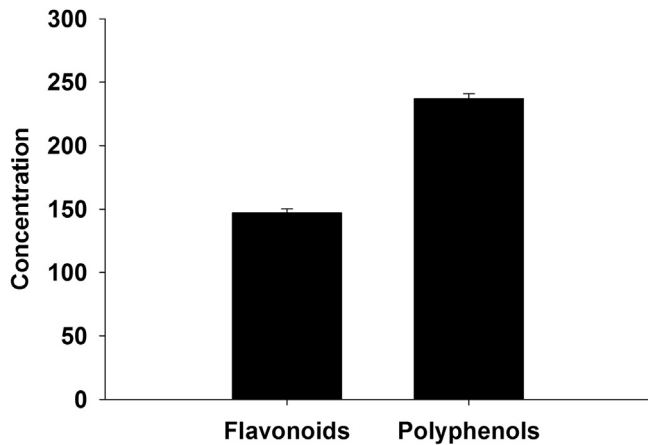


Fig. 1. Parasitemia of infected and infected-treated mice with ZSCLE at day 8 postinfection with *P. chabaudi*. \*: Significant change at  $P < .01$  between infected mice and infected-treated mice.



**Fig. 2.** Total flavonoid and phenolic compounds present in ZSCLE. Phenolic was measured as mg gallic acid equivalents per gram of the sample. Flavonoid was measured as mg quercetin equivalents per gram of the sample.

**Table 1**

Changes in leucocyte count, erythrocyte count and haemoglobin due to ZLE treatment of mice infected with *Plasmodium chabaudi* infected erythrocytes.

Parameter	Control	Infected	Infected-treated
Leucocytes $\times 10^3/\text{mm}^3$	10.2 $\pm$ 2	14.6 $\pm$ 2 <sup>a</sup>	12.8 $\pm$ 2 <sup>ab</sup>
Erythrocytes $\times 10^6/\text{mm}^3$	8.6 $\pm$ 0.7	5.6 $\pm$ 0.6 <sup>a</sup>	7.8 $\pm$ 1.3 <sup>ab</sup>
Haemoglobin (g/dL)	14.7 $\pm$ 1.6	11.1 $\pm$ 1.1 <sup>a</sup>	13.3 $\pm$ 1.4 <sup>ab</sup>
MCH (pg)	17.09 $\pm$ 1.2	19.82 $\pm$ 1.1	17.1 $\pm$ 1.2

Values are means  $\pm$  SD. a: Significant change at  $P \leq 0.05$  with respect to control group. b: Significant change at  $P \leq 0.05$  between infected mice and infected-treated mice.

(Table 1). These results are reinforced through signifying the level of mean corpuscular hemoglobin (MCH) that showed significant reduction after treatment with ZSCLE.

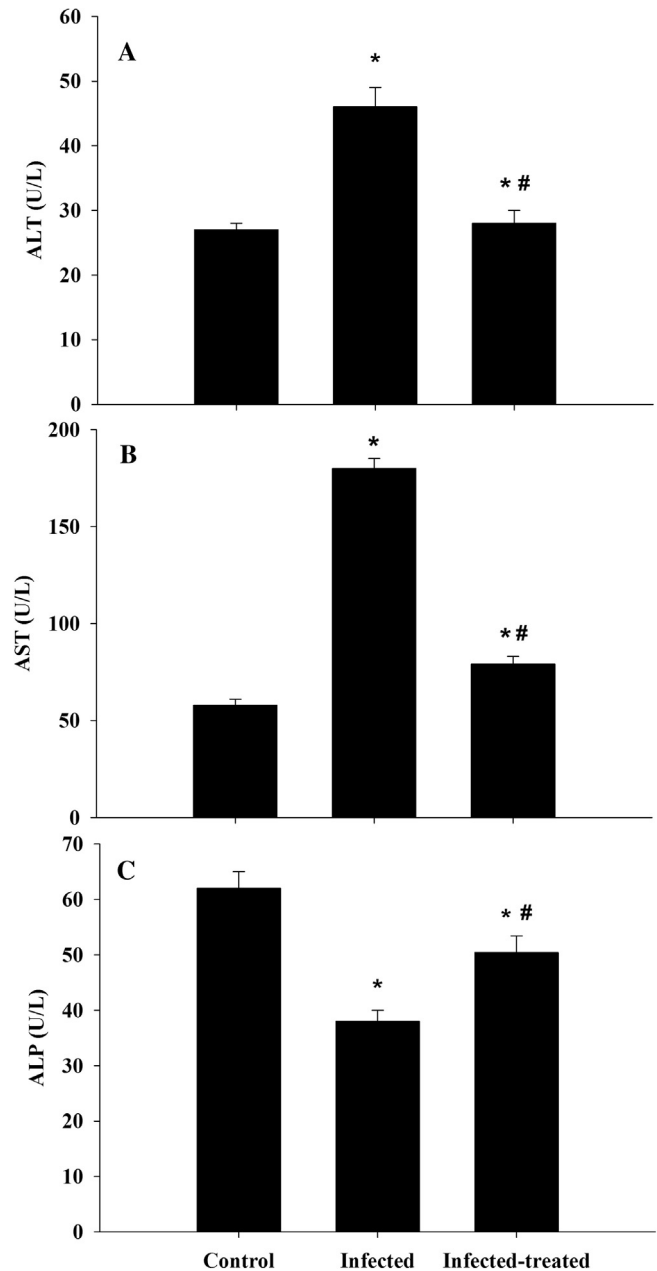
Regarding liver function tests, the levels of ALT and AST enzymes, in comparison with control group, increased significantly among mice infected with *P. chabaudi* infected erythrocytes while ALP level decreased significantly. These observations were reversed upon ZSCLE treatment, highlighting the ability of ZSCLE in relieving the burden of malaria pathogenesis (Fig. 3).

Examination of the liver sections of control (Fig. 4A) and both infected (Fig. 4B) and infected treated (Fig. 4C) animals showed the prominent inflammatory cell infiltration, increased number of van Kupffer cells and hepatocyte vacuolation in mice infected with *P. chabaudi*-infected erythrocytes. Remarkably, the liver sections of infected-ZSCLE treated mice group showed a preservation and improvement of the hepatocellular structure in comparison with that of infected mice group (Fig. 4C).

Regarding the hepatic oxidative markers, the liver oxidative damage in mice infected with *P. chabaudi* was detected through significant ( $P \leq .05$ ) alteration of malondialdehyde (MDA) and catalase compared to the control group. Considerably, the levels of hepatic MDA and catalase were restored among ZSCLE treated mice (Fig. 5).

#### 4. Discussion

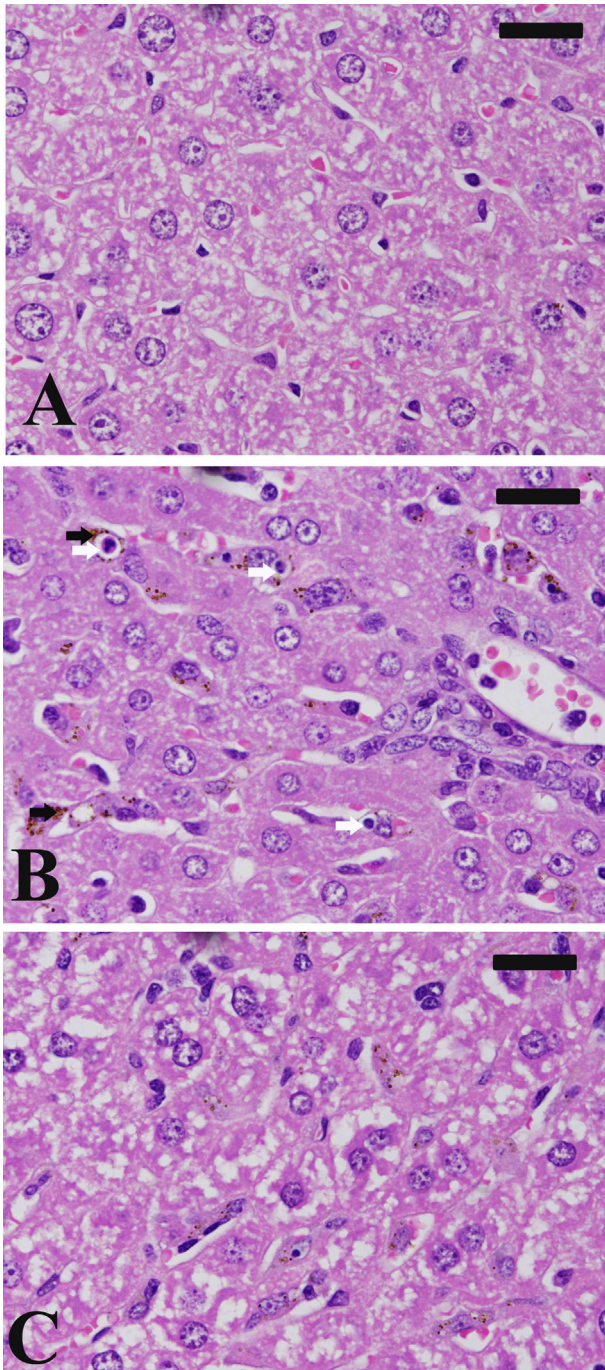
*Z. spina-christi* was studied to further confirm its antiplasmodial and antioxidant activities against malaria. The importance of exploring traditionally used herbal medicines is attributed to different factors. The emergence resistance to anti-malaria drugs, the absence of vaccination and the existing remedy effects of some plants such as *Artemisia annua* (the origin of successful anti-



**Fig. 3.** C. changes in ALT, AST and ALP due to ZSCLE treatment of mice infected with *P. chabaudi* infected erythrocytes. \*: Significant change at  $P < .01$  with respect to control group. #: Significant change at  $P < .01$  between infected mice and infected-treated mice.

malarial drug, artemisinin (Lee et al., 1989; Dua et al., 2004)), all of which are factors for initiate examination of herbal medicines validity.

This study was conducted for further validation of *Z. spina-christi* effectiveness against malaria. Measuring the parasitemia level considers being an initial validation of ZSCLE among mice infected with *P. chabaudi*. In accordance with Mishra and Bhatia study (2014), ZSCLE was able to significantly reduce the parasitemia level among ZSCLE treated infected mice. Moreover, the result of this study was in a line with recent finding of Hafiz and Mubarak (2016) that showed a remarkable reduction of parasitemia level upon *P. berghei*-induced infection. Expectedly, high parasitemic level caused discrepancies among hematological parameters that record signs of anemia, low haemoglobin level

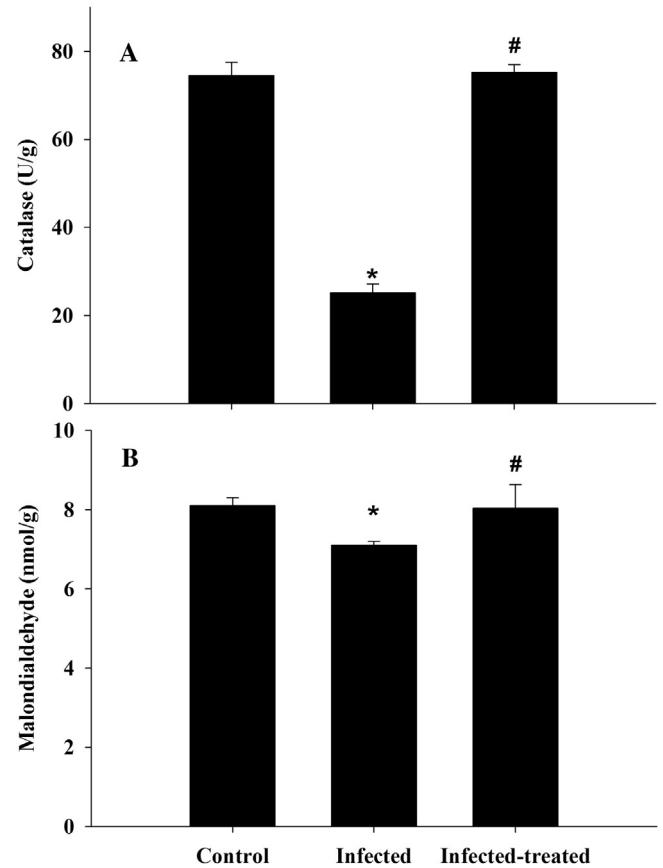


**Fig. 4.** ZSCLE ameliorates hepatic tissue damage induced by *P. chabaudi*-parasitized erythrocytes. Stained paraffin sections of mouse liver. (A) non-infected liver with normal architecture. (B) Infected liver with some inflammatory cells around the central vein. Also, malaria pigments (black arrow) and apoptotic bodies (white arrow) are prominent. (C) infected-treated liver with less induced inflammation and malaria pigments. Sections are stained with hematoxylin and eosin. Bar = 25  $\mu$ m.

and erythrocytes counts. ZSCLE, notably, was able to reverse the anemic picture and reinstate the normal levels.

The action of ZSCLE is due to the presence of several active components like essential oils, alkaloids, flavonoids and phenolic compounds (Kadioglu et al., 2016).

With regards to liver, malaria-associated liver pathogenesis has varied from elevated liver enzymes, jaundice to hepatomegaly and liver failure (Anand and Puri, 2005, Whitten et al., 2011). The levels



**Fig. 5.** Induced changes in catalase and malondialdehyde after treatment of *P. chabaudi*-infected mice with ZSCLE. \*: Significant change at  $P < .01$  with respect to control group. #: Significant change at  $P < .01$  between infected mice and infected-treated mice.

of liver function enzymes, ALT and AST, increased substantially among mice infected with *P. chabaudi* infected erythrocytes while ALP level decreased significantly. A study by (Dahiru and Obidoa, 2008) on the effect of an aqueous leaf extract of *Z. mauritiana* on chronic ethanol-induced hepatotoxicity showed significant reduction of ALT, ALP and AST enzymes among *Z. mauritiana* treated group of rat. This effect on the levels of injured tissue marker and lipid peroxidation was attributed to the presence of tannins, saponins and phenolic compounds in *Ziziphus* extract. Concomitantly, the present study demonstrated similar effect of ZSCLE on the liver functions enzymes. Furthermore, in accordance with the liver function enzymes, the histological appearance of the infected-ZSCLE treated mice group evidently verify the ability of ZSCLE to reserve the hepatocellular membrane integrity and restore the hepatic architecture. Similarly, the effectiveness of *Z. spina-christi* in treating and inhibiting hepatic fibrosis caused by carbon tetrachloride was reported by (Amin and Mahmoud-Ghoneim, 2009). Moreover, (Hafiz and Mubarak, 2016) found that upon ZSCLE treatment of liver of infected mice with *P. berghei*, the signs of inflammatory cell infiltration and hepatocyte vacuolation were significantly ameliorated. Former study by Ali and Hamed (2006) examined *Z. spina-christi* extracts role on the histopathological pictures of liver, kidney and spleen that been affected by Schistosoma infection and found that *Z. spina-christi* extracts have pronounce ameliorating effects on hepatic tissues.

The effects of ZSCLE on the oxidative marker of infected liver tissues reinforced the ameliorative role of ZSCLE against hepatic injury induced by *Plasmodium chabaudi* infected erythrocytes. This

effect evident through significant restoration of hepatic MDA and catalase levels among ZSCLE treated mice. Correspondingly, Yossef et al. (2011) study recorded the protective effect of ZSCLE among mice intoxicated with carbon tetrachloride (CCl<sub>4</sub>) (Yossef et al., 2011). Moreover, a previous study showed the amelioration effect of ZSCLE on hepatic disorders through restoring the normal levels of MDA in *Schistosoma mansoni* infected mice (El-Rigal et al., 2006). Importantly, recent study showed the protective effect of ZSCLE on hepatic and splenic tissues through reinstatement the normal levels of the oxidative markers and impeding the progression of hepatic and splenic fibrosis upon *P. berghei* induced hepatic and splenic inflammation (Hafiz and Mubarak, 2016).

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