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Original article

Indigofera oblongifolia as a fight against hepatic injury caused by murine trypanosomiasis



Mohamed A. Dkhil^{a,b,*}, Rewaida Abdel-Gaber^{a,c}, Mona F. Khalil^b, Taghreed A. Hafiz^d, Murad A. Mubarak^d, Esam M. Al-Shaebi^a, Saleh Al-Quraishy^a

^a Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia

^b Department of Zoology and Entomology, College of Science, Helwan University, Cairo, Egypt

^c Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt

^d Clinical Laboratory Sciences Department, College of Applied Medical Sciences, King Saud University, Saudi Arabia

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ABSTRACT

Trypanosoma evansi is a hazardous pathogenic parasite infecting a broad variety of livestock and affects wildlife worldwide. *Trypanosoma evansi* has gained resistance to most drugs used; therefore, it requires alternative medicines. The objective of this research was to investigate the impact of *Indigofera oblongifolia* leaf extract (IE) on *T. evansi*-induced hepatic injury.

Mice were once infected with 1000 *T. evansi*. The treated group was gavaged with 100 mg/Kg IE after infection. Histological and biochemical changes in mice hepatic tissue were studied. Also, the oxidative damage in the liver was evaluated through determining the level of glutathione (GSH), Malondialdehyde (MDA), nitric oxide (NO) and catalase (CAT) markers. IE was able to suppress the induced parasitemia due to infection. Also, IE improved the histological liver architecture. Furthermore, the liver enzymes, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activity were improved after IE mice were treated. IE protects against hepatic damage caused by trypanosomiasis in mice. Further studies are needed to isolate the active compounds in IE and to monitor these compounds' ameliorative function.

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1. Introduction

Trypanosomiasis continuous to be one of the most hazards diseases induced by protozoan parasites of the genus *Trypanosoma* that cause severe economic losses especially in Africa (Mehlhorn, 2014). Domestic and wild animals suffering from trypanosomiasis leading to weight loss, anemia, weakness and fever (Brun et al., 1998; Reid et al., 2001; Otto et al., 2009). *Trypanosoma evansi* survives in blood and causes Sura in animals (Mehlhorn, 2014). This parasite transmitted to the host by houseflies (Luckins, 1998)

and induces multiple pathological effects in many organs including spleen, liver, brain and kidney (Ghaffar et al., 2016).

Current trypanosomiasis medicines produce serious side effects and the parasite becomes resistant to the drugs used (Kirchhoff, 2009). Diminazene aceturate is commonly used to treat domestic animals infected with *T. evansi* but it causes some toxicity to the host (Do Carmo et al., 2015). It is therefore necessary to search for new safe drugs against trypanosomiasis.

Verpoorte et al. (2006) reported that about 80% of people use natural products in health care. Many plant extracts like *Azadirachta indica* (Habiba et al., 2011), *Acacia albica* (Ndidi et al., 2015), *Achyrocline satureioides* (Ritter et al., 2017), were screened for their effect against *T. evansi*. Recently, our group reported the spleen protective role of *Indigofera oblongifolia* leaf extracts (IE) against the induced injury by *T. evansi* (Dkhil et al., 2019). This plant belongs to family fabaceae and distributed in many countries of Asia and Africa. Moreover, it had been reported to induce a strong antimicrobial (Dahot, 1999) and antiparasitic (Lubbud et al., 2015) effects. Kirtikar and Basu (1984) reported that most of the plant parts were used against hepatomegaly. This study was designed

* Corresponding author at: Zoology Department, College of Science, King Saud University, P.O. Box: 2455, Riyadh 11451, Saudi Arabia.

E-mail address: mdkhil@ksu.edu.sa (M.A. Dkhil).

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to know the hepatoprotective function of IE against *T. evansi*-induced damage in mice.

2. Materials and methods

2.1. Methanolic extraction of *I. Oblongifolia*

I. oblongifolia leaves were gathered from the south region of Saudi Arabia. At a temperature of 40 °C, the gathered leaves were dried before being ground into powder. Similar to Lubbad et al. (2015), 70% methanolic extract of *I. oblongifolia* was prepared.

2.2. Infrared spectroscopy

A small amount of the sample (1 wt%) was mixed with an excess of potassium bromide (KBr) powder (99 wt%) and ground down to form a uniform consistency and then finely pulverized and put into a pellet-forming die. The instrument used for Infrared (IR) analysis is NICOLET 6700 Fourier-transform infrared spectroscopy (FT-IR) optical spectrometer from Thermo Scientific. Absorption maxima were recorded in wave numbers (cm^{-1}). Spectra were recorded from 4000 cm^{-1} to 400 cm^{-1} at standard room temperature with a resolution of 4 cm^{-1} .

2.3. Infection of animals with *T. evansi*

From the animal facility of the Zoology Department, College of Science, King Saud University, Swiss albino female mice (9–13 weeks old) were obtained. Weekly transfusion with infected blood preserved the parasite, *T. evansi*, in mice. The parasitemia was calculated according to Herbert and Lumsden (1976) by using a collected blood drop from the tail vein from mice infected with *T. evansi*.

Thirty mice, with ten animals per group, were split into 3 groups. The non-infected control group were daily gavaged with 100 μl distilled water for four days. The infected group received 1000 *T. evansi* through intraperitoneal route. Mice of IE-treated group were gavaged with the extract (100 mg/kg) 60 min after infection daily for four days, according to Dkhil et al. (2019). Blood and liver tissue from mice were collected on day 4 postinfection after being killed by CO_2 asphyxiation.

2.4. Blood analysis for leucocytes and liver function

Blood was collected from the heart of mice into heparinized tubes. Plasma was separated for measuring the liver function enzymes while we used the automatic counter (VET-530 CA Medonic; Medonic, Stockholm, Sweden), for measuring the total leucocytes count and the percentage of neutrophils and lymphocytes.

The liver enzymes, aspartic acid aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were measured in blood plasma using commercial kits (Biomerieux, Marcy l'Etoile, France).

2.5. Histology of the liver

Liver pieces were fixed in formalin (10%) then embedded in paraffin and sectioned (5 μm thickness). Sections were stained with hematoxylin and eosin (Drury and Wallington 1980).

2.6. Liver oxidative status

According to Tsakiris et al (2004), the liver homogenate was prepared using Tris-HCl and sucrose. The levels of glutathione (GSH), nitric oxide (NO) and malondialdehyde (MDA) were

determined according to Ellman (1959), Green et al. (1982) and Ohkawa et al. (1979), respectively. Also, catalase (CAT) activity was determined according to Aebi (1984).

2.7. Statistical analysis

The significance between groups was assessed by using a one-way analysis of variance and statistical comparisons were done using Duncan's test. Values were presented as mean and standard error of the mean. $P \leq 0.05$ was considered to be significant for all statistical analyses in this study.

3. Results

FTIR analysis of IE showed major bands (Fig. 1, Table S1) at 3397.97 cm^{-1} , 2365.49 cm^{-1} , 1506.08 cm^{-1} , 1116.21 cm^{-1} , 1070.87 cm^{-1} , 1014.37 cm^{-1} , 666.06 cm^{-1} , 647.46 cm^{-1} and 601.79 cm^{-1} . The bands at 1116.21 cm^{-1} , 1032.34 cm^{-1} implied to the CO stretching and C=C binding at 666.06–601.79 cm^{-1} confirming the presence of Alkene. The bands 2365.49 cm^{-1} was due to N-H stretching indicating amino salt. OH stretching was indicated by bands at 3397.97 cm^{-1} for the presence of alcohol. Also, other compounds were indicated in Table S1.

Our previous work (Dkhil et al., 2019), showed that the parasitemia was reduced by 70% after treatment of mice with IE (Fig. 2). The infection induced a significant decrease in the total number of leucocytes ($4.8 \pm 0.2, \times 10^9/\text{mm}^3$) when compared to the non-infected mice ($7.5 \pm 1.1, \times 10^9/\text{mm}^3$) (Table 1). Treatment with IE caused an elevation of the leucocytes number. Neutrophils were significantly increased by the infection while lymphocytes were significantly decreased (Table 1). *I. oblongifolia* was able to improve the altered change due to *T. evansi* infection (Table 1).

In Fig. 3, the control liver appeared with normal architecture and the hepatocytes were radially around the central vein. The infection caused marked histopathological changes in the form of inflammation, hemorrhage, hyperplasia of Kupffer cells and appearance of trypanosomes in the central vein. Improvement in the hepatic structure was observed in the liver of the infected-treated group (Fig. 3).

Fig. 4 showed that there was a marked dysfunction in the liver of *T. evansi* the infected mice. The measured enzymes activities of AST ($279 \pm 25, \text{U/L}$), ALT ($121 \pm 10, \text{U/L}$) and ALP ($95 \pm 6, \text{U/L}$) were increased when compared to that of the non-infected control group. Infected mice that were treated with IE showed improvement the enzyme activity (Fig. 4).

The infection of mice with *T. evansi* decreased the level of GSH ($1.3 \pm 0.3 \text{ nmol/g}$) in the liver homogenate compared to the control mice ($4.3 \pm 0.7 \text{ nmol/g}$) (Table 2). However, NO ($20.4 \pm 3 \mu\text{mol/L}$) and MDA ($204 \pm 60 \text{ nmol/g}$) levels were increased by the infection. In addition, CAT activity was non-significantly decreased by *T. evansi* (Table 2). IE-treated group appeared with improved oxidative status after infection (Table 2).

4. Discussion

In the present study, treatment of the infected mice with IE decreased the number of the parasites in the blood significantly when compared with their number in the control blood samples, and consequently all measured parameters were expected for improvement in the presence of IE. The protective effect can be due to the presence of IE active compounds such as phenol, quinine, saponins and coumarin (Shahjahan et al., 2005). The macroscopic examination of livers demonstrated an increase in the liver size combined with pale color in the infected animals compared to the control group. Microscopically, morphological

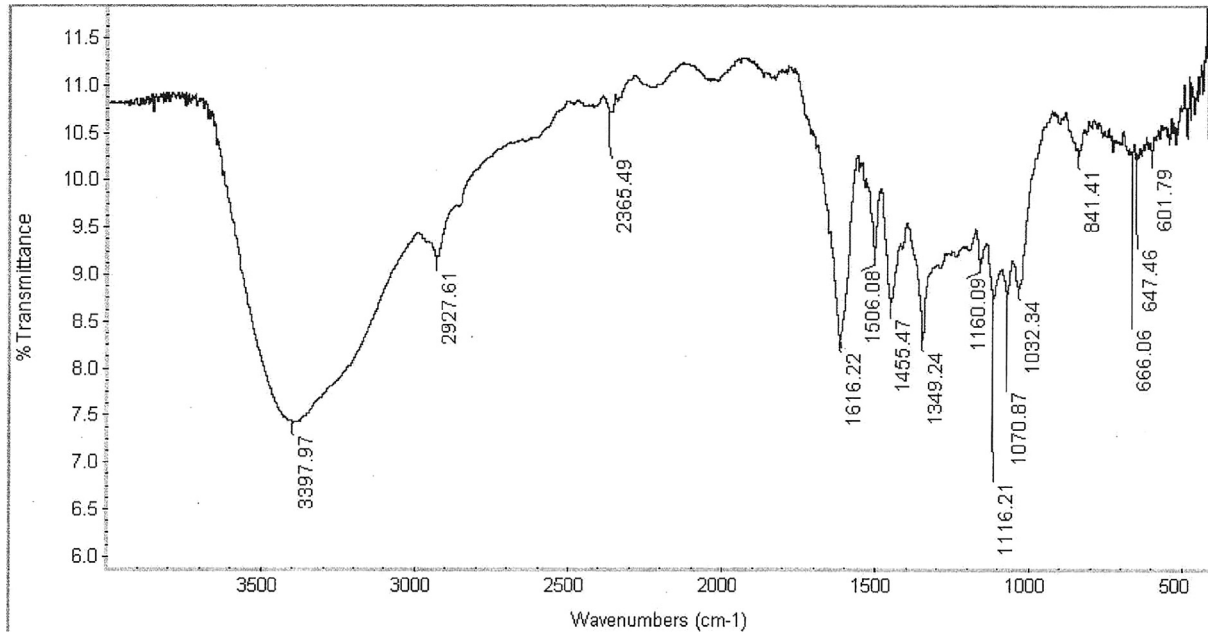


Fig. 1. Infrared spectroscopy of *I. oblongifolia* leaf extracts.

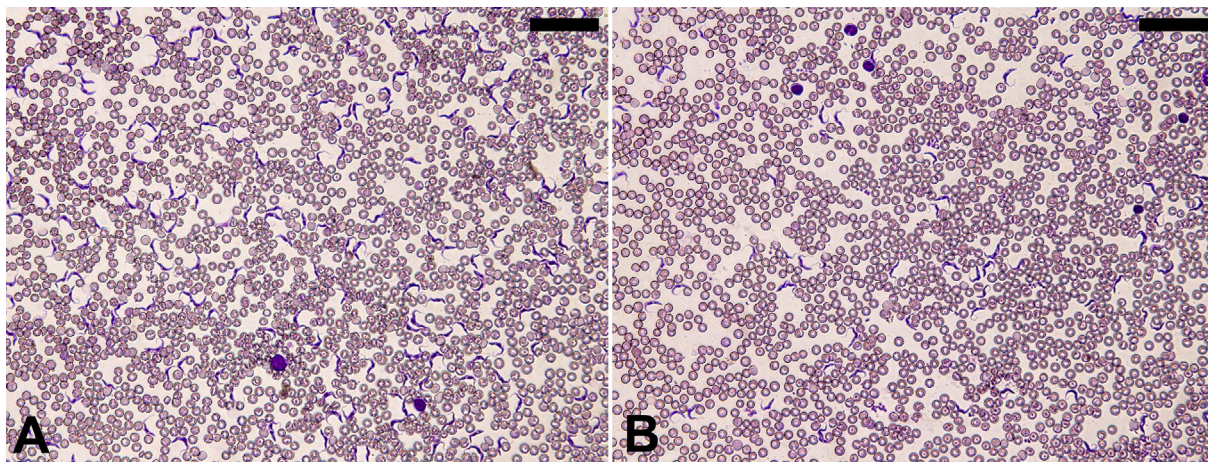


Fig. 2. Blood smear of *T. evansi*-infected (A) and infected- *I. oblongifolia* treated mice (B). Smears were stained with Giemsa. Bar = 50 μ m.

Table 1
Effect of *I. oblongifolia* on blood leucocytes infected with *T. evansi*.

Groups	Total leucocytes ($\times 10^9/\text{mm}^3$)	Neutrophils (%)	Lymphocytes (%)
Control	8.3 \pm 2	26 \pm 3	75 \pm 11
Infected	3 \pm 1 ^a	45 \pm 6 ^a	48 \pm 2 ^a
Infected-treated	5 \pm 1 ^{ab}	33 \pm 2 ^{ab}	64 \pm 3 ^b

Values are mean \pm SEM, a (control against infected), ab (infected against infected treated) are significance at $p < 0.01$.

deformities in the infected livers with the parasite resulted in indefinite intercellular spaces between hepatocytes, and the hepatic cells lost their definite polygonal shapes. Moreover, cells and membranes were very much destructed, cytoplasm was much vacuolated, and dilation of sinusoids packed them with blood cells and the parasites. Also, infected livers have shown congestion, hemorrhage and depletion of fats in their cells, in addition to the aggregation of inflammatory cells around the central canal and the portal regions, and masses of the parasites were observed in the

central vein and the inflamed sinusoid regions. Our histopathological results agreed with those obtained by Bal et al., 2012 and Al-Otaibi et al (2018) on mice infected with *T. evansi*, and by Biswas et al., 2001 on infected rats with the parasite. Our results assured the previous results that trypanosomiasis induces severe irreversible destructions and alterations that end in cell apoptosis (Biswas et al 2001). Most of the histopathological alterations of the infected hepatocytes could be due to the lowered blood levels of glucose, which result in anemia, which in turns leads to cell starvation and anoxia (Biswas et al., 2001; Bal et al., 2012). As the parasite utilizes oxygen to reproduce, the host tissue cells become deprived of oxygen, resulting in hypoxic condition accompanied with the destructive changes in the liver and other organs (Ghaffar et al., 2016; Al-Otaibi et al., 2018, Dkhil et al., 2019). It was also reported that the toxins released by the parasite into the plasma and tissue fluid could have a significant role in histopathological alterations in the liver (Ghaffar et al., 2016); they induce hyper-lysosomal secretions, where lysosomes burst resulting in cell autolysis, which initiates necrosis of the liver cells (Biswas et al., 2001). On the other hand, treatment of the infected

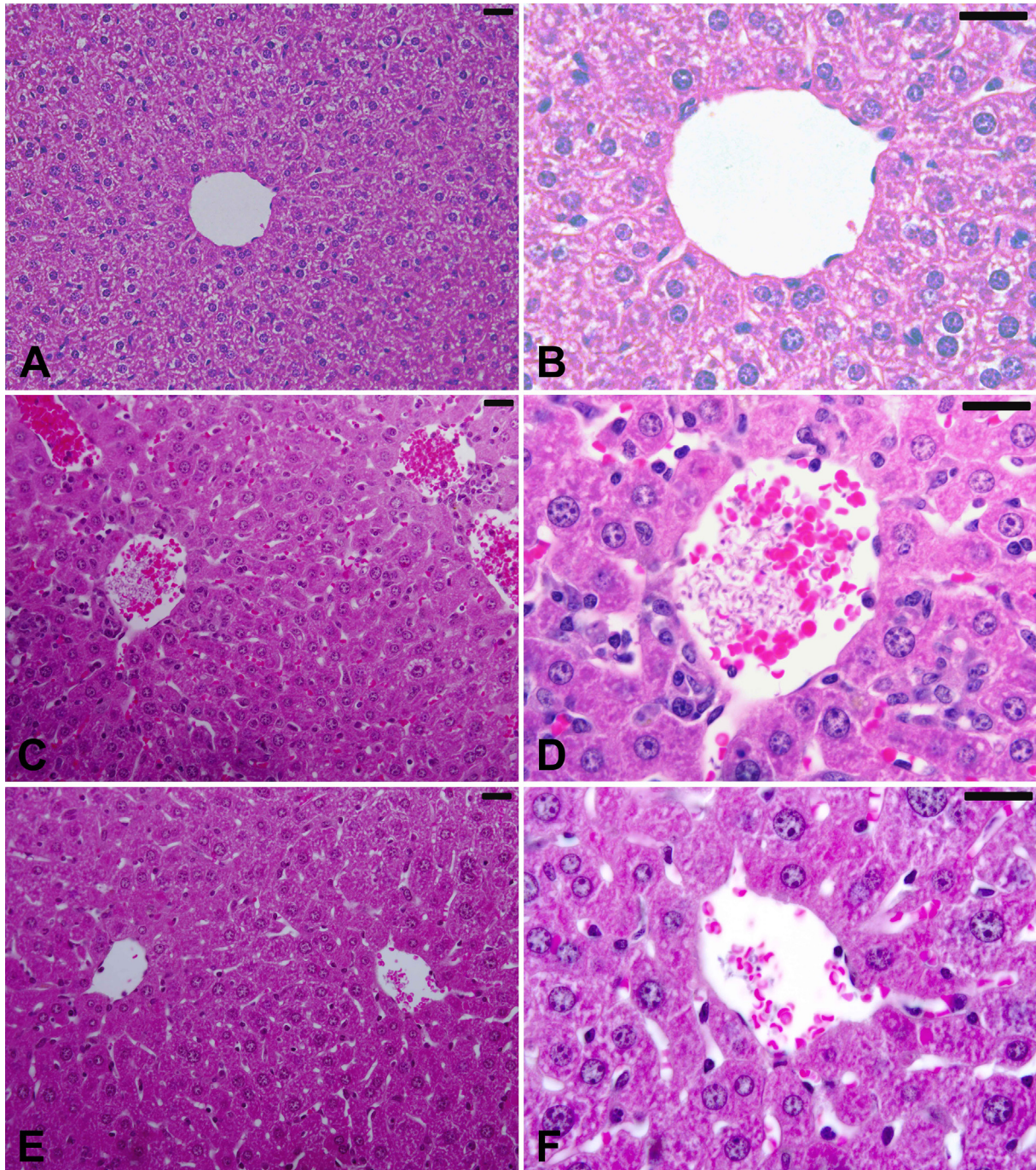


Fig. 3. Effect of *I. oblongifolia* on liver histology of infected with *T. evansi*. (A,B), control mice with normal histology. (C,D), Infected liver with inflammation, hemorrhage, hyperplasia of Kupffer cells and many trypanosomes in the central vein. (E,F), Infected-treated liver with improved histological structure.

mice with IE has significantly reversed the severe destructive histopathological conditions induced by the parasite in the infected group.

Anemia is the major clinical symptom of trypanosomiasis (Suliman and Feldman, 1989), and it is in turn accompanied with leucopenia and thrombocytopenia (Anosa, 1988), and leucopenia is in turn associated with lymphocytopenia (Al-Otaibi et al., 2018). The hematological results of our study presented a significant leucopenia associated with lymphocytopenia in infected mice compared to the control group. Depletion of leucocytes in the infected mice is associated also with alterations in the spleen, the major lymphoid organ (Dkhil et al., 2019). It was

reported that the lymphoid system develops focal hemorrhage, necrosis (Dkhil et al., 2019), and infiltration of leucocytes, followed by depletion of the lymphocytes in both infected sheep with *T. evansi* and dogs with *T. brucei*. Studies proved improvement of the immune lymphoid tissues against trypanosomiasis by variable plant extracts (Mahassni and Khudauardi, 2017; Al-Otaibi et al., 2018). Interestingly, treatment of animals with IE restored leucopenia induced by the parasite infection, and this recovery could refer to the protective effect of IE on the spleen, as an immune system organ against *T. evansi* infection (Dkhil et al., 2019). Restoration of leucocytes' count in the treated mice could refer to enhancement and strength of the spleen induced by IE

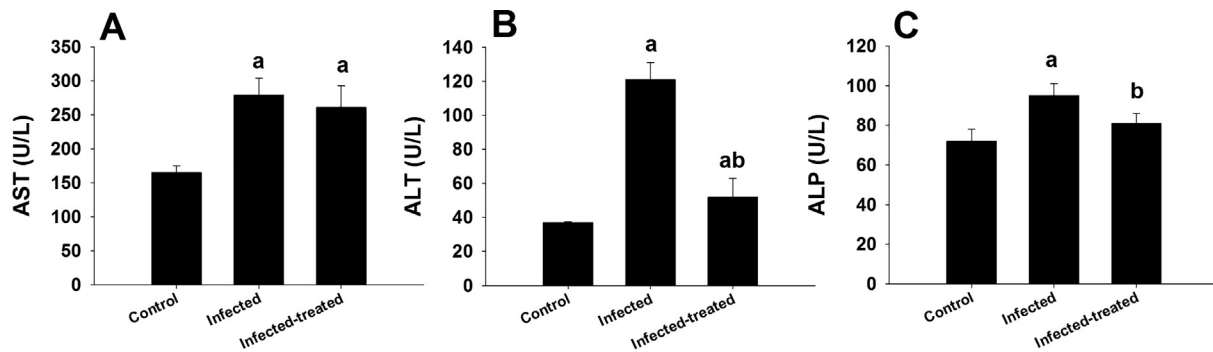


Fig. 4. Effect of *I. oblongifolia* on liver enzymes, AST, ALT and ALP in the blood plasma of *T. evansi* infected mice.

Table 2

Effect of *I. oblongifolia* on *E. evansi* infected mice liver GSH, NO, MDA and CAT.

Groups	GSH (nmol/g)	NO (μ mol/L)	MDA (nmol/g)	CAT (U/g)
Control	4.3 \pm 0.7	6.3 \pm 0.6	85 \pm 20	7.4 \pm 2
Infected	1.3 \pm 0.3 ^a	20.4 \pm 3 ^a	204 \pm 60 ^a	6.6 \pm 0.1
Infected-treated	3.5 \pm 1.8 ^b	13.7 \pm 2 ^{ab}	167 \pm 20 ^a	7.3 \pm 0.4 ^b

Values are mean \pm SEM, a (control against infected), ab (infected against infected treated) are significance at $p < 0.01$.

(Mahassni and Khudauardi, 2017; Dkhil et al., 2019). Our results agree with the studies of Mahassni and Khudauardi, (2017) and Al-Otaibi et al. (2018), who reported that treatment with *Lepidium sativum* seed extract (LSSE) improves leucopenia and lymphocytopenia induced by *T. evansi* in mice.

In the present study, the parasite infection has significantly altered levels of both the hepatic redox and oxidative stress agents; it lowered the levels of GSH and CAT enzyme significantly compared to the control livers. On the other hand, infection significantly elevated the levels of hepatic MDA and NO compared to the control group. It has been reported that trypanosome infection induces production of ROS in erythrocytes, which in turn elevates lipid peroxidation, and thus alters antioxidants' levels (Wolkmer et al., 2009). Additionally, during infection, the products of oxidative burst from immune system cells initiates haemolysis via destruction of the fatty acids in the plasma membrane of erythrocytes (Taiwo et al., 2003). Therefore, in our study, the oxidative products represented in MDA and NO, could attribute in inhibiting the antioxidant protection activity "represented in GSH and CAT enzyme" of the red blood cells in the infected mice against oxidative destruction of the cell membranes (Wolkmer et al., 2009). Our results on the oxidative stress parameters during infection agrees with those obtained by Dkhil et al., 2019, on the other hand, our study demonstrated that treatment of infected animals with IE have restored the oxidative stress and altered antioxidant activity induced by the parasite infection. And the presented results are consistent with those reported by a previous study of our team, who proved the antioxidant activities of IE in the spleen against the oxidative damage induced by *T. evansi* (Dkhil et al., 2019). Moreover, our results demonstrated significant elevated levels of the liver function markers, ALT, AST and ALP in infected mice, which may refer to destruction of hepatic tissue post parasite infection (Nwoha et al., 2013), and those findings agree with those reported by Sivajothi et al. (2015) and Al-Otaibi et al. (2018) and were consistent also with both the hematology and oxidative stress results of the present study. On the other hand, treatment of the parasite infected mice with IE retained the corresponding liver enzymes to the normal levels, and this assures the protective role of IE against the hepatotoxicity induced by trypanosomiasis.

Our results agree with those of Al-Otaibi et al., 2018 who presented the protective role of LSSE against toxic effects induced by *T. evansi* infection in the liver cells of mice, they reported the ability of LSSE to limit the destruction of the liver cells via enhancing and normalizing the levels of hepatic enzymes (Sakran et al., 2014).

Our study interestingly confirmed the protective role of IE that was reported on a recent study made by our team on the splenic tissues of mice infected by *T. evansi* against the hepatotoxicity induced by *T. evansi*. Interestingly, the present study proved the protective role of IE and its antioxidant activity against the destructive changes induced in the liver tissues of the infected mice with the parasite. Therefore, natural plant extracts could be considered as powerful drugs to treat and/or reduce the destructive effects of trypanosomiasis in the host tissues.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.sjbs.2019.11.038>.

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