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ORIGINAL ARTICLE

Histopathological Studies on the Curative Role of *Mentha longifolia* in *Trypanosoma evansi* Experimentally Infected Rats

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Abstract

Trypanosomiasis is an important protozoan disease of domestic animals and man. One of the important pathogenic trypanosomes in animals is *Trypanosoma evansi*; the causative agent of Surra that is highly fatal to a number of domesticated mammals such as camels. *Mentha longifolia* is a plant that has been used as traditional medicine to treat some parasitic and microbial diseases in different countries. This study was conducted to evaluate the antitrypanosomal activity of the leaf ethanolic extract of M. longifolia against experimentally induced *T. evansi* infection in rats. A total of 16 male albino rats were allocated into four groups of four rats each; uninfected control group (CG), plant extract group (PG), trypanosome infected group (TG), and infected treated group (plant extract + trypanosome) (PTG). Blood and tissue samples were collected from rats on the 35th day after *T. evansi* infection and treatment. Hematological results obtained from *T. evansi*-infected rats showed microcytic hypochromic anemia with a significance of ($P< 0.05$). There was a significant ($P< 0.05$) increase in the total leukocytic count (TLC) and number of neutrophils, eosinophils, and monocytes in TG) compared to those in CG, PG, and PTG. Histopathological examination revealed necrosis, hydropic degeneration and liver apoptosis, hemorrhage, edema, inflammatory cell infiltration of kidney, demyelination of brain, lymphocytic granuloma of lung. Results of the present study showed that the antitrypanosomal activity of *M. longifolia* extract was not effective in vivo, despite its high antitrypanosomal activity in vitro.

Keywords Antitrypanosomal activity, Histopathological examination, *Mentha longifolia*, Rats, *Trypanosoma evansi*

1. Introduction

Trypanosoma. evansi infection is a major concern in camel breeding and development in Africa and Asia **(Ashour et al. 2013; Eyob and Matios 2013).** It is the primary cause of "*Surra*" trypanosomiasis, comprising one of the major veterinary problems worldwide **(Aradaib and Majid 2006; OIE 2018)**. In general, trypanosomiasis is a serious protozoal infection affecting a wide range of domestic animals as well as wild animals. *T. evansi* infection results in degenerative changes in various vital body organs such as the spleen, liver, heart, lung, brain and kidney. *Trypanosoma. evansi* attacks different camel species leading to high economic losses due to expensive drugs, high mortality rates, weight loss, decreased animal productivity **(Eyob and Matios 2013),**

abortion and stillbirths (**Djamila et al. 2019).** Trypanosomiasis has been described as either chronic or acute and symptomatic or asymptomatic in both experimental and natural infections **(Ashour et al. 2013).** Both the incidence and severity of the disease are generally higher in domestic animals than in wild animals (**Morrison 1981)**. Necropsy findings reveal gross and irreversible histopathological changes in the liver, lung, spleen and kidney **(Ashour et al. 2013)**.

The efficiency of available drugs in the treatment of trypanosomiasis is limited by several factors. Such as unavailability, treatment failure and treatment period. Treatment may be clinically curative without complete elimination of the parasite, along with a possibility of relapses, increase in parasite resistance and high cost. On the other hand, the toxicity and efficacy of the drug may vary from one species to another **(Don and Chatelian 2009; Muhammad and Helen 2017)**. Several studies have reported the use of some plants as a basis for traditional treatment for different types of trypanosomiasis **(Aderbauer et al. 2008; Peter et al. 2009)** and have also been reported to be trypanocidal against *T. evansi* **(Adeiza et al. 2009; Muhammad and Helen 2017).** The wild mint (*Mentha longifolia*), grows in different areas and extensively in Mediterranean region, Europe, North Africa, and Australia. **(Gulluce et al. 2007).** It is also used in Iran as a traditional medicine for treating digestive disorders **(Zargari 1990).** This plant possesses medicinal and aromatic properties with a peppermint-scented aroma **(Omidbaigi 2005).** It is used in different industries such as food and pharmaceutical industries, specifically in cosmetology. In traditional folk medicine, different components of *M. longifolia* have been reported to exert antimicrobial, carminative, stimulant, and antispasmodic activities and have also been used for the treatment of different diseases such as headaches and digestive disorders (**Naghibi et al. 2010).** The extract of *Mentha* species possesses antimicrobial, anti-inflammatory, and antioxidant properties, and it is also considered as a fungicidal agent **(Mimica-Dukic et al. 2003; Gulluce et al. 2007; Mkaddem et al. 2009**). There is also extensive research on the hepatoprotective, nephroprotective, and neuroprotective functions of this plant **(Ayshath et al. 2016)**. The present investigation was conducted to evaluate the trypanocidal activity of the leaf ethanolic extract of *M. longifolia* against *T. evansi* in experimentally infected rats.

2. Material and Methods

2.1. Plant material

M. longifolia was collected from different locations on the northern west coastal zone of Egypt. The plant was identified as *Mentha longifolia* L. family Lamiaceae (The wild mint) at the Medicinal and Organic Plants Department, the Desert Research Center, Egypt.

2.2. Preparation of extracts

The leaves of the plant were air-dried to a constant weight and then grounded into powdered form using an electric blender (Kenwood®). The powdered sample was extracted using a Soxhlet extractor. The ethanolic extract was prepared by soaking 25 g of the powdered plant material in a solution of 200 ml of 95% ethanol, followed by extraction with petroleum ether to de-fat the sample, and then filtered using Whatman no. 1 filter paper. Next, the filtrate was gently evaporated on a water bath set at 45°C for dryness. The obtained residue was weighed and used for administration by dissolving in Tween 80 (0.3% v/v) and then stored in airtight containers in a refrigerator until needed.

2.3. Phytochemical screening of extracts

The contents of phenols, saponins, tannins, flavonoids, and alkaloids in the crude extract were determined as described previously **(Parekh et al. 2005).**

2.4. Trypanosome propagation

T. evansi was isolated from naturally infected camels at El-Bassatein abattoir (Cairo, Egypt). It was identified and maintained in the laboratory by continuous passage in white Swiss mice of mixed sexes (weighing 20–25 g) to produce parasitemia of approximately 10^5 parasites/ml. Mice were injected intraperitoneally with 0.01 ml of blood containing approximately 1×10^3 trypanosome cells. Then, a preparation of blood at 40× magnification was used to estimate parasitemia. The trypanosome count was determined using the "rapid matching" method described by **Herbert and Lumsden** (**1976).**

2.5. In vitro test for trypanocidal activity of plant leaf extract

The in vitro antitrypanosomal activity of the plant extract was evaluated in duplicates in 96-well microtiter plates. Briefly, 40µl of blood infected with *T.* evansi containing approximately 50-100 parasites per field were incubated with aliquots of 10µl of extract in a concentration of (10.45) in the wells of microtiter plates. As control, phosphate-buffered saline was used in the wells of microtiter plates instead of 10µl of the extract as described earlier **(Atawodi et al. 2009).** Thereafter, the motility of parasites was observed under the microscope (40×) after 5 min of incubation at 37°C. The parasites were observed every 10 min for a total duration of 60 min. A decrease in motility and a reduction of parasite count were considered as a measure of the antitrypanosomal activity of the extract **(Barghash 2016).**

2.6. Safe dose and acute toxicity

Lethal dosage test was conducted as described previously **(Lorke 1983).** Two groups of plant extracts were assessed for the two most suitable concentrations based on the results of microtiter plates, as follows: the first for the minimal dose (LD50) and the second for the maximal dose (LD90). The results demonstrated that the two selected doses of the plant extract were safe for rats. The combination of these results made LD90 of plant extract the best option for processing in vivo study, which resulting in disappearance of *T. evansi*.

2.7. In vivo assay and experimental design

This experiment was conducted according to the common ethical guidelines on the treatment and handling of laboratory animals. A total of 16 male rats (*Rattus norvegicus*) with an average body weight of 200g were used in this study. Mice were first allowed to adapt to the surroundings and then divided into four groups of four rats each; uninfected control group (CG): 4 rats were injected intraperitoneally with 0.5ml of phosphate-buffered saline; plant extract group (PG): 4 rats were injected intraperitoneally with 0.5ml of plant extract; trypanosome infected group (TG): 4 rats were injected intraperitoneally with 0.1 ml of blood collected from infected mice containing 3×10^5 trypanosomes; and infected treated group (plant extract + trypanosoma group) (PTG): 4 rats were injected intraperitoneally with 0.1 ml of blood collected from infected mice containing 3×10^5 trypanosomes +0.5ml of plant extract. On the six-day post infection and after three days of extract administrations, the parasitaemia level of rats in the controls and extract group were checked three times a week by wet blood film prepared from tail blood at x40 magnification. Rats were observed daily for clinical signs which include weight loss, facial edema, ocular discharges without any mortality recorded along the experiment. After slaughtering the post mortem lesions include splenomegaly, hepatomegaly, lung hemorrhage, congested heart in both *trypanosma* infected group and infected treated group (plant extract + *trypanosom*a group). On the 35th day blood samples were collected through heart puncture, and tissue samples were collected from the kidney, liver, heart, brain, spleen, and lung. Blood samples were divided in two parts; the first tube contained EDTA for the estimation of hematological parameters, the second tube was plain and clean for serum separation; blood was allowed to coagulate and then centrifuged at 3000 rpm for 20 min at 37°C, after which the separated serum was collected in a clean Eppendorf tube for the estimation of biochemical parameters **(Tomita et al. 2001; Roberts et al. 2006).**

Hematological parameters were evaluated according to (**Feldman et al. 2000),** and the biochemical analysis was conducted spectrophotometrically using commercial kits supplied by Biodiagnostic company® (all manual instructions were followed).

2.8. Histopathological examination

The collected tissue samples were rapidly fixed in 10% neutral buffered formalin solution, washed, dehydrated in ascending grades of ethyl alcohol, cleared in methyl benzoate, and then embedded in paraffin wax. Several paraffin sections of 3–5 micron thickness were obtained and stained with hematoxylin and eosin as described earlier **(Bancroft and Gamble 2008)**.

2.9. Statistical analysis

The measured parameters were expressed as mean ± standard deviation. Data were analyzed using the SPSS program version 24, in which the mean values of different statistical parameters were compared by one-way ANOVA, and post hoc differences between mean values were estimated using Tukey's multiple comparison HSD test. $P < 0.05$ was considered to indicate statistical significance.

3. Results

Regarding the in vitro antitrypanosomal activity, *M. longifolia* extract. It exhibited high activity against *T. evansi* parasites as observed by the disappearance of the parasite within 10–45 min of incubation. In the in vivo experiment, all groups experimentally infected with *T. evansi* developed parasitemia of approximately 10^6 parasites/ml at day 3 post infection (day 0 for beginning treatment). After treatment with the extract, a decrease in parasitemia and a complete parasite clearance were observed on the 3rd day after treatment, which reappeared at 7 days post treatment. Treatment with the second dose (after 14 day from the first dose) produced the same result with no difference, and on the 35th day after treatment, the parasite was detected in high levels. Regarding the clinicopathological results shown in **Table (1),** there was a significant (P< 0.05) decrease in RBC count, mean corpuscular hemoglobin (MCH) level, mean corpuscular hemoglobin concentration (MCHC), hemoglobin (Hb) level, and packed cell volume (PCV) in TG, PG, and PTG compared to those in CG, in TG compared to those in PG, PTG, and a significant (P< 0.05) decline in RBC count, Hb level, and PCV between PTG and PG. Mean corpuscular volume (MCV) exhibited a significant (P< 0.05) increase in TG only when compared with CG and a nonsignificant change in PG or PTG. In contrast, there was a significant (P< 0.05) increase in the total leukocytic count (TLC) and number of neutrophils, eosinophils, and monocytes in TG compared to those in CG, PG, and PTG. However, significant (P< 0.05) leukopenia, neutropenia, eosinopenia, and monocytepenia were observed in PG and PTG compared with CG and TG. A significant (P< 0.05) elevation in TLC was also recorded between PTG and PG, whereas the lymphocyte count was significantly (P< 0.05) decreased in TG compared to that in CG, PG, and PTG and significantly increased in PG and PTG compared to that in CG and in PTG compared to that in PG.

Table 1. Comparison between the haematological parameters of the studied groups at 35th day, values are means ± SD

Parameters	CG	TG	PG	PTG
RBCs (×10⁶/µ)	5.27±0.22[d]	1.24±0.03[a]	2.99±0.04[a,b]	2.56±0.09[a,b,c]
Hb (g/dl)	15.58±0.43[d]	2.20±0.24[a]	6.30±0.11[a,b]	5.55±0.21[a,b,c]
PCV (%)	30.98±0.82[d]	11.88±0.15[a]	16.50±0.08[a,b]	15.43±0.31[a,b,c]
MCV (fl)	58.76±1.14[d]	96.03±3.67[a]	55.23±0.48	60.28±1.39
MCH (pg)	29.54±0.43[d]	17.74±1.48[a]	21.09±0.38[a,b]	21.71±1.33[a,b]
MCHC (%)	50.27±0.43[d]	18.54±2.26[a]	38.18±0.64[a,b]	35.99±1.53[a,b]
TLC(×103/µl)	5.19±0.22[d]	13.86±0.79[a]	2.79±0.47[a,b]	4.05±0.12[a,b,c]
N(×103/µl)	2.48±0.28[d]	10.68±0.61[a]	0.40±0.08[a,b]	0.35±0.05[a,b]
L(×103/µl)	1.65±0.13[d]	1.13±0.10[a]	1.97±0.06[a,b]	3.17±0.06[a,b,c]
E(×103/µl)	0.51±0.08[d]	1.20±0.03[a]	0.10±0.01[a,b]	0.16±.06[a,b]
M(×103/µl)	0.56±0.04[d]	0.86±0.08[a]	0.33±0.07[a,b]	0.37±0.05[a,b]

CG: Control group, **TG:** Trypanosome group**, PG:** Plant extract group, **PTG:** Plant Extract + Trypanosoma group.
[a](significant with CG), [b](significant with TG), [c](significant with PG), [d](significant between the 4 groups), considered significant when P<0.05.

Regarding the biochemical results shown in **Table (2),** there was a significant (P<0.05) decline in the levels of TP, Alb, A/G, TAC, CAT, glucose, cholesterol, and HDL/LDL-cholesterol in TG, PG, and PTG compared to those in CG, in TG compared to those in PG and PTG, and in PTG compared to those in PG. In contrast, the levels of Glob, liver enzymatic activities (AST, ALT, and ALP), kidney function parameters (urea and creatinine (Cr)), triglycerides, and phospholipids were significantly increased (P<0.05) in TG, PG, and PTG compared to those in CG, in TG compared to those in PG and PTG, and in PTG compared to those in PG.

Table 2. Comparison between the biochemical parameters of the studied groups at 35th day, values are means ±SD

Parameters	CG	TG	PG	PTG
TP (g/dl)	7.39±0.12[d]	6.09±0.07[a]	6.05±0.03[a]	6.82±0.07[a,b,c]
Alb (g/dl)	4.58±0.17[d]	1.14±0.06[a]	3.35±0.11[a,b]	2.59±0.13[a,b,c]
Glob (g/dl)	2.81±0.08[d]	4.95±0.09[a]	2.71±0.12[b]	4.24±0.15[a,b,c]
A\G	1.63±0.10[d]	0.23±0.10[a]	1.24±0.10[a,b]	0.61±0.05[a,b,c]
TAC (Mm/L)	1.93±0.06[d]	0.76±0.02[a]	1.16±0.03[a,b]	0.91±0.03[a,b,c]
CAT (U/L)	446.50±1.73[d]	212.50±9.57[a]	393.50±3.42[a,b]	308.75±8.54[a,b,c]
AST (U/L)	25.01±0.82[d]	45.01±1.15[a]	28.01±0.82[a,b]	32.25±1.71[a,b]
ALT (U/L)	27.51±2.08[d]	49.01±1.15[a]	35.50±1.29[a,b]	39.25±0.96[a,b]
ALP (U/L)	23.27±2.06[d]	47.01±1.15[a]	28.45±0.53[a,b]	34.25±1.71[a,b]
Urea (mg/dl)	20.86±0.13[d]	36.47±0.14[a]	24.62±0.28[a,b]	28.64±0.22[a,b,c]
Cr (mg/dl)	0.67±0.07[d]	1.59±0.05[a]	0.89±0.03[a,b]	1.16±0.04[a,b,c]
Glucose (mg/dl)	122.25±1.71[d]	67.35±1.95[a]	91.29±2.79[a,b]	73.05±2.18[a,b,c]
Total lipids (mg/dl)	587.75±6.95	581.25±6.08	580.01±8.04	569.50±2.38
Triglycerides (mg/dl)	144.75±2.22[d]	192.75±3.40[a]	152.50±2.08[a,b]	164.25±1.29[a,b,c]
Phospholipids (mg/dl)	153.01±2.58[d]	204.51±1.29[a]	161.01±1.15[a,b]	172.25±1.71[a,b,c]
Cholesterol (mg/dl)	145.01±2.58[d]	92.01±1.63[a]	133.25±2.75[a,b]	116.50±1.29[a,b,c]
HDL-c (mg/dl)	44.75±0.96[d]	29.01±2.58[a]	40.75±0.96[a,b]	36.75±0.96[a,b,c]
LDL-c (mg/dl)	100.25±1.71[d]	63.01±2.58[a]	92.51±1.91[a,b]	79.75±1.71[a,b,c]

CG: Control group, **TG:** Trypanosome group**, PG:** Plant extract group, **PTG:** Plant Extract + Trypanosoma group.
[a](significant with CG), [b](significant with TG), [c](significant with PG), [d](significant between the 4 groups), considered significant when P<0.05.

Histopathological examination:

Liver: The liver tissue of CG **(Fig. 1A)** showed normally arranged hepatocytes around central veins with normal portal areas, but in PG **(Fig. 1B),** the liver tissue showed areas of degenerative changes with coagulative necrosis, congestion of blood vessels especially central vein and sinusoids. Hepatic sinusoids were dilated and had proliferated Kupffer cells. On the other hand, in TG **(Fig. 1C),** the liver tissue showed areas of severe infiltration of lymphocytes and vacuolar degeneration of hepatocytes. Liver apoptosis was detected in PTG **(Fig. 1D).**

Spleen: Histopathological examination of the spleen revealed normal architecture in the red and white pulps.in CG **(Fig. 2A),** whereas in PG **(Fig. 2B),** the spleen showed edema, congestion, and depletion of white pulp. In TG **(Fig. 2C),** the spleen showed hyperplasia of lymphoid follicles with clear appearance of giant cells and hemorrhage. Hemosiderosis of spleen was clearly observed (brown coloration) in PTG **(Fig. 2D).**

Kidney: In CG **(Fig. 3A),** the kidney tissue showed normal appearance of cortex and medulla. In PG **(Fig. 3B),** the histopathological examination revealed hypercell-ularity of glomeruli with intramedullary hemorrhage, slight edema, necrosis of tubules and severe infiltration of chronic inflammatory cells. In TG **(Fig. 3C),** the findings were intertubular infiltration of chronic inflammatory cells, hemorrhage, edema, necrosis of tubules, and atrophy of glomeruli. In PTG **(Fig. 3D),** the kidney tissue showed atrophy of glomerular tuft and hyaline matrix within glomeruli, hyaline cast inside the tubule, and necrosis of tubules.

Heart: The control group **(Fig. 4A)** showed more or less normal heart muscles, the heart tissue of rats in PG **(Fig. 4B)** showed edema, hemorrhage, and slight necrosis of muscles. In TG **(Fig. 4C),** the findings were myocardiolysis, slight congestion, and infiltration of inflammatory cells with degenerative changes of muscles (swelling of muscle). In PTG **(Fig. 4D),** the heart tissue showed edema and granulation of the cytoplasm of muscles with clear appearance of necrosis of muscle (Zenker's necrosis).

Lung: In CG **(Fig. 5A),** microscopic examination of the lung tissue showed alveolar emphysema and hemorrhage. In PG **(Fig. 5B),** the lung tissue showed catarrhal bronchitis and hyperplasia of epithelial lining, peribronchial edema with slight lymphocytic infiltration, and atelectasis. In TG **(Fig. 5C),** the findings were catarrhal bronchitis and bronchiolitis, edematous fluid with slight desquamated cells within the bronchial lumen, peribronchial edema with severe infiltration of lymphocytic cells, and alveolar emphysema. In PTG **(Fig. 5D),** there were lymphocytic granuloma and compensatory alveolar emphysema in the lung tissue.

Brain: The brain tissue of rats in PG **(Fig. 6B)** showed perineural edema, congested blood vessels, and slight demyelination of brain cells. In TG **(Fig. 6C),** the findings were perineural edema, chromatolysis of neurons, and vacuolation. In PTG **(Fig. 6D),** the brain tissue showed neuronophagia, perineuronal edema, and increased demyelination of brain cells.

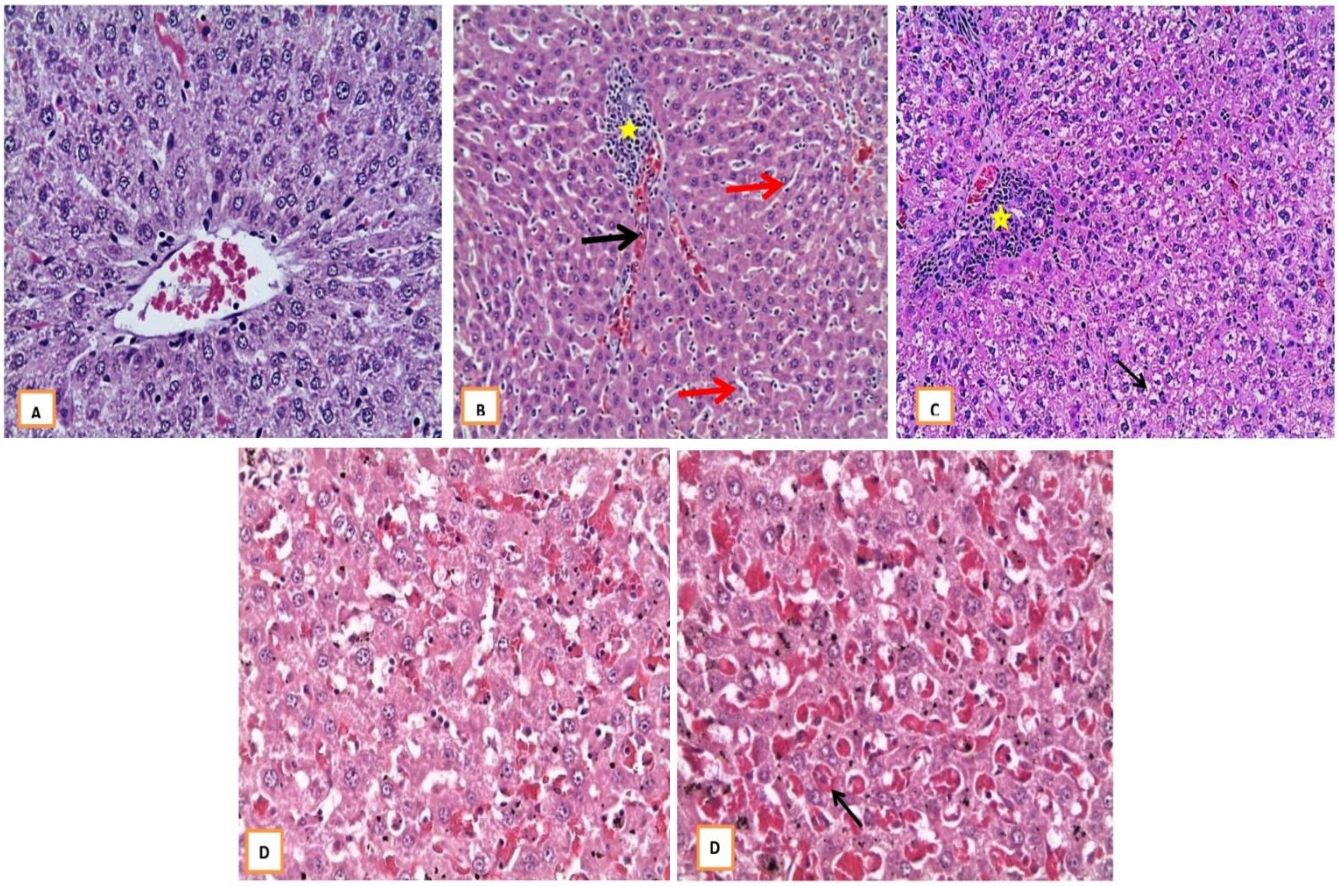

Fig. 1. Liver of a rat; **(A)** normal liver showing plates of hepatocytes. **(B)** area of coagulative necrosis **(star),** congestion **(black arrow),** and hepatic sinusoid dilatation **(red arrow). (C)** liver with area of coagulative necrosis **(star),** congestion and vacuolar degeneration of hepatocytes **(black arrow). (D)** liver showing apoptosis **(black arrow)** and high power magnification,

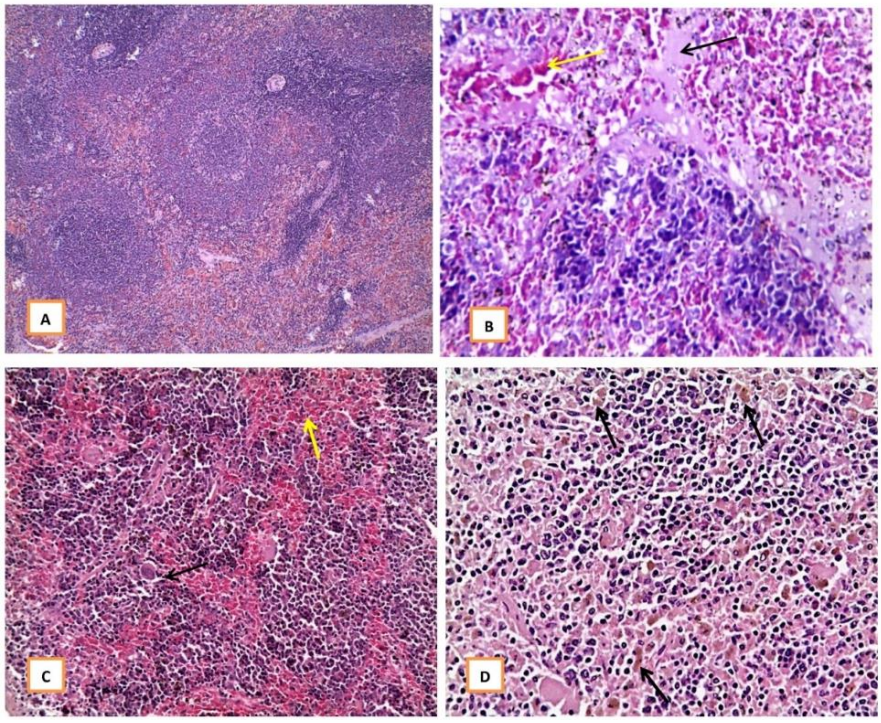

Fig. 2. Spleen of a rat; **(A)** panoramic view of the spleen. **(B)** edema (black arrow) and congestion **(yellow arrow). (C)** hyperplasia of the lymphoid follicle **(black arrow),** hemorrhage **(yellow arrow),** and depletion of the white pulp. **(D)** haemosiderosis of the spleen, brown color **(black arrow).**

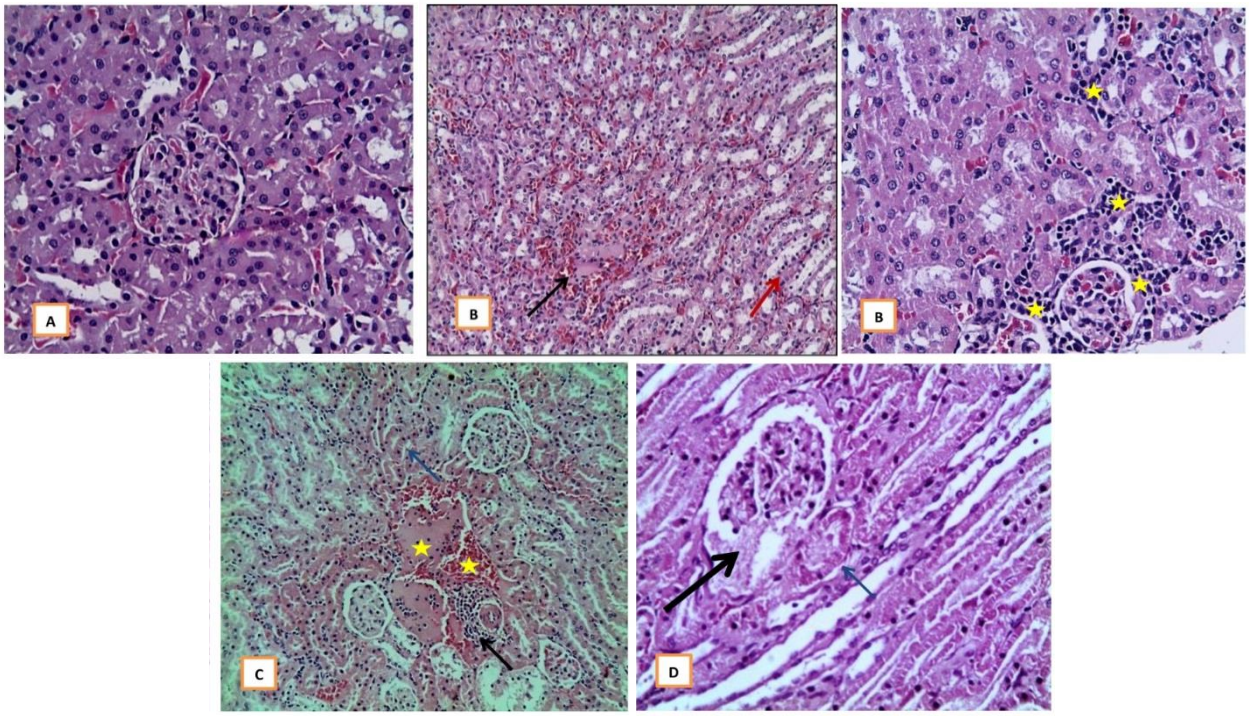

Fig. 3. Kidney of a rat; **(A)** control rat showing the glomeruli of the kidney, **(B)** intramural hemorrhage **(black arrow)** and slight edema, necrosis of the tubules **(red arrow), (B)** infiltration of the chronic inflammatory cells **(stars), (C)** infiltration of the chronic inflammatory cells **(black arrow),** hemorrhage and edema **(star),** and necrosis of tubules **(black arrow), (D)** atrophy of the glomerular tuft and hyaline matrix within the glomeruli **(thick arrows)** and hyaline cast in the tubules **(blue arrow).**

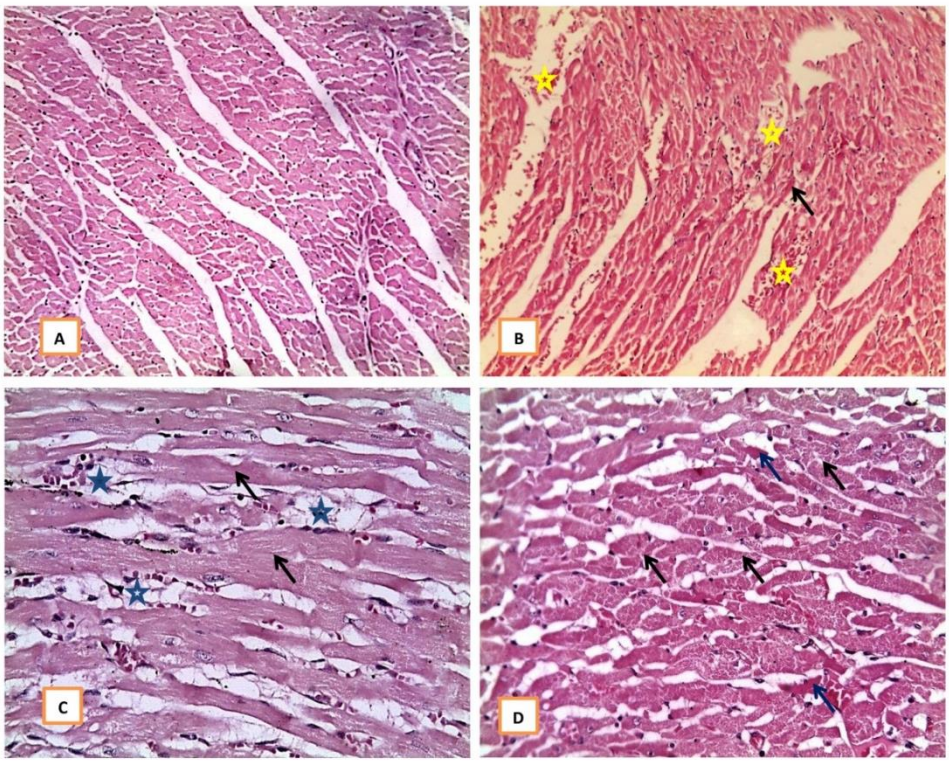

Fig. 4. Heart of a rat; **(A)** control rat. **(B)** edema and hemorrhage **(yellow star)** and necrosis of the cardiac muscles **(black arrow). (C)** myocardiolysis **(blue stars)** and swelling of the muscles **(black arrow)** and loss of striation. **(D)** granulation of the cytoplasm of the muscles **(black arrow),** and necrosis of the muscles **(blue arrow),** and slight edema**.**

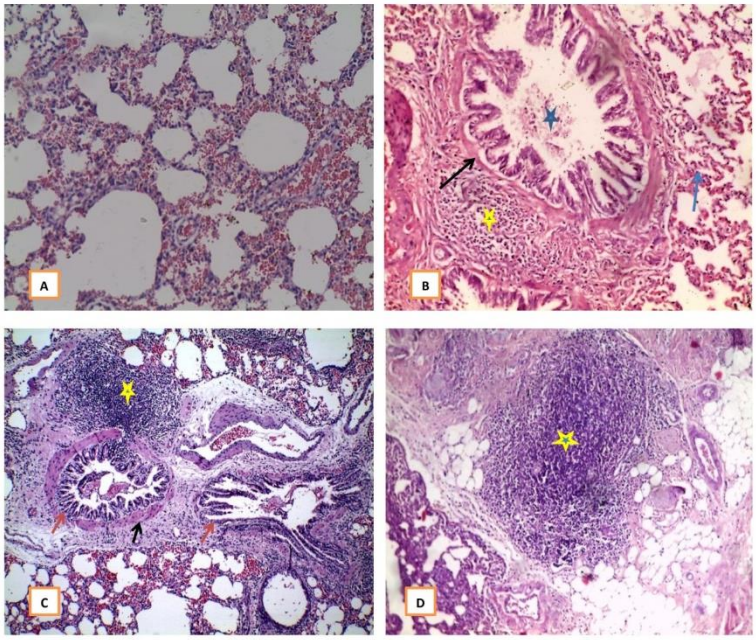

Fig. 5. Lung of a rat; **(A)** control rat with alveolar emphysema and hemorrhage, **(B)** catarrhal bronchitis **(black arrow),** desquamated epithelial cells and edematous fluid inside the lumen **(blue star),** peri-bronchial edema, lymphocytic infiltration **(yellow star)** and atelectasis **(blue arrow), (C)** catarrhal bronchitis and bronchiolitis **(red arrow)** with massive lymphocytic infiltration **(yellow star)** and alveolar emphysema, **(D)** lymphocytic granuloma **(yellow star),** and compensatory alveolar emphysema**.**

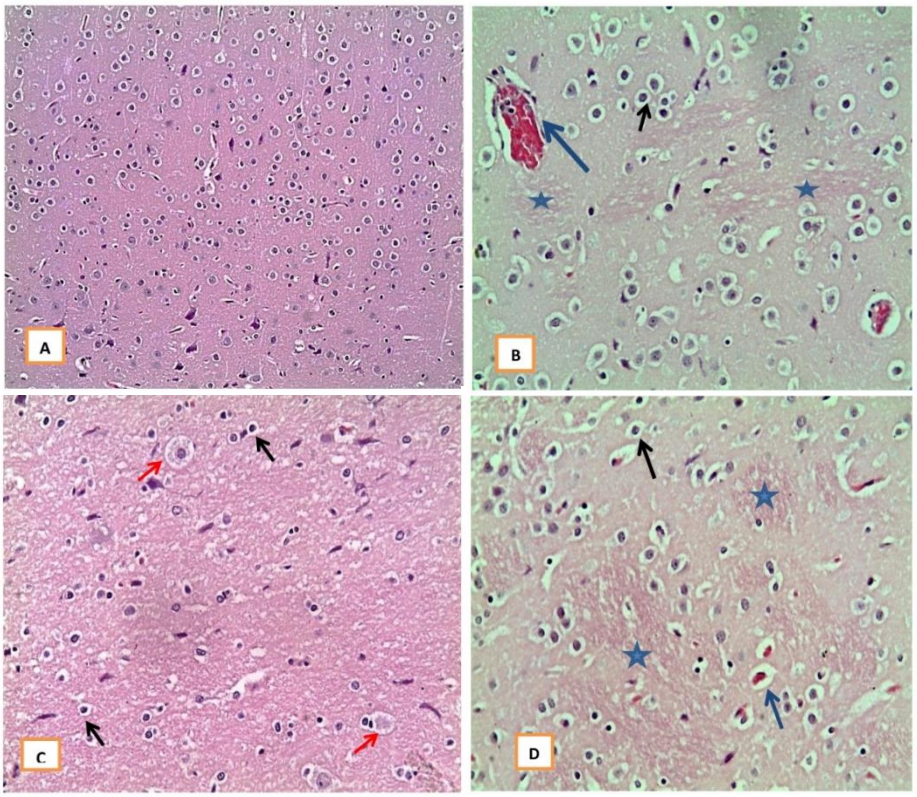

Fig. 6. Brian of a rat; **(A)** control rat. **(B)** perineural edema **(black arrow),** congested blood vessels **(blue arrow),** and slight demyelination of the brain **(star). (C)** perineural edema **(black arrow),** chromatolysis of the neurons **(red arrow). (D)** neuronphaia **(blue arrow),** perineural edema **(black arrow),** and increase in demyelination of the brain **(stars).**

4. Discussion

T. evansi (*surra* disease) is a major parasitic disease affecting different camels' species especially in the high camel breeding countries, resulting in severe economic losses. To avoid such economic losses, it is important to analyze the pathological and clinicopathological changes associated with trypanosomiasis, which could help in developing effective therapeutic interventions **(Abd El-Baky and Salem 2011** and **Ahmadi-hamedani et al. 2014).** Results of the present study concerning the hematological changes were consistent with the common belief that *T. evansi* infection is always accompanied by anemia, which is defined etiologically as hemolytic and morphologically as microcytic hypochromic **(Abd El-Baky and Salem 2011; Ahmadi-hamedani et al. 2014)**. *M. longifolia* contains different ketones, saponins, flavonoids, monoterpenes, and tannins, which are responsible for the different biological and pharmacological activities of this plant **(Ayshath et al. 2016).** Several studies have demonstrated that plants with high antitrypanosomal activity in vitro may have no activity in vivo **(Freiburghaus et al. 1996** and **Atawodi et al. 2003).** To obtain additional evidence for the antitrypanosomal activity of the tested material, an in vitro experiment was conducted in this study, followed by an in vivo experiment. Results showed that the antitrypanosomal activity of *M. longifolia* extract was not effective in vivo, despite its high antitrypanosomal activity in vitro, which is in agreement with previous findings **(Freiburghaus et al. 1996; Brghash 2016; Alhaji 2014)**, which clearly showed that a plant with no trypanocidal activity in vivo may have high trypanocidal activity in vitro and vice versa. This may be due to the peculiarities in the metabolic disposition of the plant's chemical constituents. The hematological characteristics of the parameters evaluated in the four study groups are summarized in Table 1. A significant decrease was observed in RBC count, PCV, Hb level, MCH and MCHC, in TG, PG, and PTG compared to that in CG, whereas MCV showed a significant ($P < 0.05$) increase in TG only. This finding is consistent with previous reports **(Barghash 2016; Razin, 2017).** Infected rats with high MCV values and low MCHC values indicated the presence of macrocytic hypochromic anemia. The mechanical destruction of RBCs, may be attributed to the metabolic activities of

the parasite in the blood stream and the release of trypanosomal sialidase enzyme, which is responsible for hydrolyzes the sialic of RBCs membrane leading to destruction of RBCs and erythrogensis inhibition **(Ahmadi-hamedani et al. 2014; Darwish et al. 2019).** More ever the innate immunity of the host itself has an important role of this anemia by more than one way, as hem dilution due to removal of large amounts of RBCs in haemal lymph nodes and spleen by mononuclear phagocytic system activation or through oxidative damage of RBCs **(Zewdu et al. 2018).** Monocytosis, neutrophilia, eosinophilia, and subsequent leukocytosis observed in this study are primarily linked to the immune response of the host against the parasite through mononuclear phagocytic system activation. In addition, neutrophilic leukocytosis may be associated with disease stress, resulting in the stimulation of endogenous corticosteroid production; these results are similar to the findings of a previous study **(Ahmadi-hamedani et al. 2014).** Eosinophilia is a characteristic feature of different parasitic infections due to the hypersensitivity reaction **(Abd El-Baky and Salem 2011).** In contrast, significant ($P< 0.05$) leukopenia, neutropenia, eosinopenia, and monocytopenia were observed in PG and PTG compared to those in CG and TG in the present research. These results indicate that the levels of the tested lipids Chol, TG, HDL, LDL, and VLDL generally tended to increase in response to *M. longifolia* treatment compared with negative control animals. However, the levels of the tested lipids Chol, TG, HDL, LDL, and VLDL generally tended to reduce in untreated animals. **Abimbola et al. (2013)** demonstrated that *T. brucei* infection causes a significant decrease in the serum concentrations of Chol, HDL, TG, and LDL. Although hypoglycemia is one of the major markers of trypanosomal infection, resulting in increased catabolism of lipids and cholesterol for energy requirements **(Vigueira et al. 2012)**, in the same manner, *T. evansi* hinders glucose release from the gluconeogenic pathways of the host as it also consumes most of the host's blood glucose to meet its energy requirements **(Mbaya et al. 2014).** The present study also showed a decrease in serum lipid levels caused by hypertriglyceridemia and hypocholesterolemia in *T. evansi* experimentally infected rats. Hypertriglyceridemia was observed due to the inhibition of lipoprotein lipase enzyme by pro-inflammatory cytokines **(Darwish et al. 2019).** Furthermore, **Adam et al. (2009); Bala et al. (2012)**; **Biu et al. (2013)** explained the reduction of serum triglycerol and cholesterol levels as a result of trypanosomal continuous utilization of lipid molecules from the blood stream as an important source of energy. On the other hand, the hypertriglyceridemia and hypercholesterolemia in group D of wild mint closely attached with defective plasma triglyceride degradation probably leads to unavailable free fatty acid for importation into hepatocytes despite elevation of serum triglyceride **(Igbokwe et al. 2009**). The elevated activity levels of the enzymes ALT and AST in the control and treated animals could be attributed to necrosis and inflammation in the liver, heart, and kidney tissues of the host or probably due to host destruction of trypanosomes as reported previously **(Abubakar et al. 2005; Takeet and Fagbemi 2009).** Moreover, there was a significant difference in the levels of serum globulin, total bilirubin, total protein, and albumin in infected and treated animals compared to those in the nontreated noninfected (N-N) group, which could be due to the difference in the antitrypanosomal activities of the selected plant extract. The elevated levels of urea and creatinine in *T. evansi*-infected rats could be due to the destruction of kidney cells, resulting in the failure of kidneys to excrete creatinine during the disease course **(Abd El-Baky and Salem 2011; Boniface et al. 2011).**

The mean values of Tp, Alb, and A/G ratio decreased significantly in case of rats treated with *M. longifolia* extract as compared with the healthy rats. The histopathological findings observed in this study support the above mentioned clinicopathological changes based on the appearance of cell necrosis, fibrosis, infiltration of the chronic inflammatory cells, edema, and loss of structure in the examined organs. The spleen serves as the first-line defense mechanism (**SivaJothi et al. 2013)**. Gross lesions of the examined organs were splenomegaly, hepatomegaly, and congested lung and heart. The gross lesions observed in the present study were also consistent with those reported by **SivaJothi et al. (2013 and 2015)** who recorded splenomegaly in rabbits and rats infected with *T. evansi*. Enlargement of spleen might be due to increased activity of the phagocytic system for the elimination of trypanosomes, paleness of visceral organs, congested lungs, and hepatomegaly. The initial changes in the spleen of rats in trypanosome or the other tested group may be due to the immediate hypersensitivity to the parasite, as reported previously (**Uche and Jones 1993)**. Splenic hemorrhage with pronounced histiocyte aggregation leading to the formation of multinuclear giant cells in the spleen and also hemosiderosis were observed in experimentally *T. evansi*-infected Swiss albino mice **(Bal et al. 2012).**

The liver tissue revealed vacuolar degeneration of hepatocytes. The histopathological changes included congestion of central vein and sinusoids, hemorrhages, massive infiltration of chronic inflammatory cells with clear appearance of apoptotic cells, and also slight hepatic necrosis, which are consistent with those of a previous study (**Razin 2017).** In the present study, the lung was severely affected with characteristic serous pneumonia and granulomatous reaction that clearly appeared with catarrhal bronchitis and bronchiolitis with alveolar emphysema, as reported in a previous study **(Bal et al. 2012).**

The brain tissue showed mild degenerative changes such as meningeal blood vessel congestion. These changes are due to the release of toxic substances by *T. evansi* **(Bal et al. 2012)**. Moreover, the other lesions, including neuronophagia, chromatolysis of neurons, and demyelination of brain cells, may be due to the nervous manifestation recorded in rats infected with *T. evansi*. The kidney tissue showed necrosis of tubules, congestion, edema, and infiltration of chronic inflammatory cells. Glomeruli were shrunken and degenerated. Moreover, the kidney tissues in other groups were characterized by hyper cellularity of glomeruli and intramedullary hemorrhage. All these changes in the kidney tissues are attributed to the toxins released by *T. evansi* and also due to the accumulation of immune complexes, resulting in the loss of structure and impairment of kidney function; these results are in agreement with those reported by several authors **(Morrison et al. 1981; Uche and Jones 1992; Ngeranwa et al. 1993; Biswas et al. 2001).** The myocardium revealed mild degenerative changes, interstitial edema, hemorrhage, and Zinker's necrosis of muscles. The degenerative changes in the heart muscle may be due to hypoglycemia and anemia as reported previously **(Biswas et al. 2001; Bal et al. 2012; SivaJothi et al. 2015).**

5. Conclusion

T. evansi infection is always associated with several histopathological and clinicopathological changes. Although *M. longifolia* extract has demonstrated a wide range of antibiotic activity against various yeasts and bacteria and also exhibited potential in vitro antitrypanosomal activity in the present study, it was not found to be efficacious in vivo even at the safest doses administered to rats. The activity of liver enzymes may be elevated, which may be due to the compounds menthone and pulegone. As the process of oven-drying decreases the amount of these compounds in the plant, it is recommended that this plant be cooked or oven-dried to make it safer before consumption **(Peyman et al. 2013).** In this study the *Mentha longifolia* considered toxic for rats through histopathological examination. Moreover, the results of the present study demonstrated that the antitrypanosomal activity of *M. longifolia* extract was not effective in vivo, despite its high antitrypanosomal activity in vitro in experimentally infected rats**.**

6. Conflict of interest

Authors declared no conflict of interests exist.

7. References

Abd El-Baky AA, Salem SI (2011). Clinicopathol-ogical and cytological studies on naturally infected camels and experimentally infected rats with Trypanosoma evansi. World Applied Sciences Journal 14(1): 42-50.

Abimbola AM, Baba IA, Yenusa EZ, Omanibe SJ, Oladimeji IH (2013). Anti–trypanosomal effect of *Peristrophe bicalyculata* extract on *Trypanosoma brucei brucei* infected rats. Asian Pacific Journal of Tropical Biomedicine 3: 523-531.

Abubakar A, Iliyasu B, Yusuf AB, Igweh AC, Onyekwelu NA, Shamaki BU, Afolayan DO, Ogbadoyi EO (2005). Antitrypanosomal and haematological effects of selected Nigerian medicinal plants in Wistar rats. Biokemistri 17: 95-99.

Adam S, Barde N, Abenga JN, Useh NM, Ibrahim NDG, Esievo KAN (2009). Experimental *Trypanosoma brucei* infection-induced changes in the serum profiles of lipids and cholesterol and the clinical implications in pigs. Cell. Anim. Biol. 3(2): 15-20.

Adeiza AA, Maikai VA, Hassan FB (2009): Phytochemical screening and evaluation of some medicinal plants for their in vitro activities on Trypanosoma evansi. Journal of Medicinal Plants Research 3: 315-318.

Aderbauer B, Clausen PH, Kershaw O, Melzig MF (2008). In vitro and in vivo trypanocidal effect of lipophilic extracts of medicinal plants from Mali and Burkina Faso. Journal of Ethnopharmacology 119: 225-231.

Ahmadi-hamedan M, Ghazvinian K, Darvishi MM (2014). Asian Pacific Journal of Tropical Biomedicine 4(9): 743-745. https://doi.org/10.12980/APJTB.4.2014APJTB-2014-0053.

Alhaji UI, Samuel NU, Aminu M, Chidi AV, Umar ZU, Umar UA, Adewale BM (2014). *In vitro* antitrypanosomal activity, antioxidant property and phytochemical constit-uents of aqueous extracts of nine Nigerian medicinal plants. Asian Pacific Journal of Tropical Disease 4: 348-355.

Aradaib IE, Majid AA (2006). A simple and rapid method for detection of *Trypanosoma evansi* in the dromedary camel using a nested polymerase chain reaction. Kinetoplastid Biology and Disease 5: 2.

Ashour AA, Abou El-Naga TR, Barghash SM, Salama MS (2013). Trypanosoma evansi: Detection of *Trypanosoma evansi* DNA in naturally and experimentally infected animals using TBR1 & TBR 2 primers. Experimental Parasitology 134: 109-114.

Atawodi SE, Ogunbusola F (2009). Evaluation of antitrypanosomal properties of four extracts of leaves, stem and root barks of *Prosopis africana* in laboratory animals. Biokemistri 21(2): 101-108.

Atawodi S, Bulus T, Ibrahim S, Ameh D, Nok A, Mamman M, Galadima M (2003). In vitro trypanocidal effect of methanolic extract of some Nigerian savannah plants. African Journal of Biotechnology 2: 317-321.

Ayshath Afroos Shahana AR, Karale S, Kamath JV (2016). Nephroprotective role of Mentha longifolia against cyclophosphamide induced nephrotoxicity in rats: A biochemical and histopathological study. Internati-onal Research J. of Pharmacy 7(6): 77-82. https:// dx.doi.org/10.7897/2230-8407.07668

Bala AY, Adamu T, Abubakar U, Ladan MJ (2012). Amelioration of trypanosome-infection-induced alterations in serum cholesterol, triglycerides and proteins by hydro-ethanolic extract of Waltheria indica in Rats. Research Journal of Parasitology 6: 127-135.

Bal MS, Singla LD, Kumar H, Vasudev A, Gupta K, Juyal PD (2012). Pathological studies on experimental Trypanosoma evansi infection in Swiss albino mice. Journal of Parasitic Diseases: Official Organ of the Indian Society for Parasitology 36(2): 260-264. https://doi.org/10.1007/s12639-012-0120-5

Bancroft JD, Gamble M (2008). Theory and practice of histological techniques. In: Swisher, B. (ed.) Microorganisms, Churchill Livingstone, Philadelphia.

Barghash SM (2016). Evaluation of in vitro and in-vivo activities of some medicinal plants against trypanosomiasis. International Journal of Advanced Research 4: 1169-1178.

Biu AA, Lawan BB, Joshua L, Mohammed K, Jude O, Nelson OA (2013). In vitro assessment of trypanocidal activity of aqueous extract of Citrullus lanatus (Cucurbitaceae) (Thunb.) leaf and its effects on the haematological parameters of Trypanosoma brucei infected albino rats. Biokemistri 25(3): 127-132.

Boniface A, Augustine I, Ikechukwu I, Paschal U (2011). Prevalence and haemato-biochemical parameters of trypanosome-infected pigs at Nsukka, Nigeria. Comparative Clinical Pathology 20(1): 15-18.

Darwish AA, Tahoun EAE, Donia GR, Mohammed RS (2019). Clinicopathological studies and new markers for Trypanosoma evansi in experimentally infected rats. Advances in Animal and Veterinary Sciences 7(11): 977-985. http://dx.doi.org/10.17582/journal.aavs/2019/7.11.977.985

Djamila Boushaki D, Adel A, Dia ML, Büscher P, Madani H, Brihoum BA, Sadaoui H, Bouayed N, Kechemir Issad NK (2019). Epidemiological investigations on Trypanosoma evansi infection in dromedary camels in the South of Algeria. Heliyon 5: e02086.

Don R, Chatelain E (2009). Drug discovery for neglected diseases: View of a public–private partnership. In: Paul, M.S. Ed. Antiparasititc and Antibacterial Drug Discovery: From Molecular Targets to Drug Candidates. 1st ed, Wiley-VCH, Weinheim Verlag GmbH and Co. KGaA, pp. 33-43.

Eyob E, Matios L (2013). Review on camel trypanosomosis (surra) due to Trypanosoma evansi: Epidemiology and host response. Journal of Veterinary Medicine and Animal Health 5(12): 334-343. https://doi.org/10.5897/JVMAH2013.0236

Feldman BF, Zinkl JC, Jain NC (2000). Schalm's Veterinary Hematology' 5th edn. Lippincott Williams & Wilkins, Philadelphia, London.

Freiburghaus F, Kaminsky R, Nkunya MH, Brun R (1996). Evaluation of African medicinal plants for their *in vitro* trypanocidal activity. J. Ethnopharmacology 55: 1-11.

Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*. Food Chemistry, Google Scholar 103: 1449-1456.

Herbert WJ, Lumsden WHR (1976). Trypanosoma brucei: A rapid matching method for estimating the host's parasitaemia. Experimental Parasitology 40: 427-431.

Igbokwe IO, Buratai LB, Ubah UL, Aromnde A, Igbokwe NA (2009). Serum and hepatic lipid levels in rats infected with *Trypanosoma brucei*. Comparative Clinical Pathology 18: 191-195.

Lorke D (1983). A new approach to practical acute toxicity testing. Archives of Toxicology 54: 275-287. Losos, G.

Mbaya AW, Kumshe HA, Dilli, HK (2014). Serum biochemical changes in dromedaries experimentally infected with Trypanosoma evansi and treated with melarsenoxyde cysteamine hydrochloride 84(4): 377-385.

Morrison WIM, McLntyre WIM (1981). Bovine trypanosomiasis in Diseases of Cattle in the Tropics. In: Ristic, M.R.a.M., W.I. (Ed.), The Hague, Martinus-Nijhoff, pp. 469-497.

Fathuddin MM, Inabo HI (2017). In vitro antitrypanosomal potential of chloroform leaf extract of Punica granatum L. on Trypanosoma brucei brucei and Trypanosoma evansi. Microbiology Research 8(1): 24-27.

Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, Mathieu F, Romdhane M (2009). Chemical composition and antimicrobial and antioxidant activities of Mentha (*longifolia* L. and viridis) essential oils. Journal of Food Science 74: M358-M363.

Mimica-Dukić N, Bozin B, Soković M, Mihajlović B, Matavulj M (2003). Antimicrobial and antioxidant activities of three *Mentha* species essential oils. Planta Medica 69: 413-419. PubMed Google Scholar.

Naghibi F, Mosaddegh M, Mohammadi Motamed M, Ghorbani A (2010). Labiatae family in folk medicine in Iran: From ethnobotany to pharmacology. Iranian Journal of Pharmaceutical Research: 63-79.

Ngeranwa JJ, Gathumbi PK, Mutiga ER, Agumbah GJ (1993). Pathogenesis of *Trypanosoma* (*brucei*) *evansi* in small east African goats. Research in Veterinary Science 54: 283-289.

OIE (World Organisation for Animal Health) (2018). Chapter 3.1.21. *Trypanosoma evansi* Infection (Surra). Manual of Diagnostic Tests and Vaccines for Terrestrial Animals.

Omidbaigi R (2005). Production and Processing of Medicinal Plants, Behnashr Press, Mashhad (in Persian).

Parekh J, Nair R, Chanda S (2005). Preliminary screening of some folklore medicinal plants from Western India, for potential antimicrobial activity. Indian Journal of Pharmacology 37: 406-409.

Peter O, Magiri E, Auma J (2009). Evaluation of in vivo antitrypanosomal activity of selected medicinal plant extracts. Journal of Medicinal Plants Research 3: 849-854.

Mikaili P, Mojaverrostami S, Moloudizargari M, Aghajanshakeri S (2013). Pharmacological and therapeutic effects of *Mentha longifolia* L. and its main constituent, menthol. Ancient Science of Life 33(2): 131-138.

Razin AR (2017). Antitrypanosomal Activity of Some Medicinal Plants and Plasma Proteins on Trypanosoma evansi Infection, Institute African Researches and Studies, Cairo University.

Roberts W, McMillin G, Burtis C, Bruns D (2006). D.E. In: Burtis, C.A. Ashwood, E.R. Bruns Eds: Reference Information for the Clinical Laboratory. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, vol. 56, Elsevier Inc., 2251-2318.

Sivajothi S, Rayulu VC, Sujatha K, Sudhakara Reddy BS (2015). Study of histopathological changes in experimental *Trypanosoma evansi* infected rats. Proceedings of the Zoological Society, Springer 68: 112-115.

Sivajothi S, Rayulu V, Sujatha K, Reddy SB, Amaravathi P (2013). Histopathological observations in rabbits experimentally infected with *Trypanosoma evansi*. Journal of Advanced Veterinary Research 3: 122-126.

Takeet MI, Fagbemi BO (2009). Haematological, pathological and plasma biochemical changes in rabbits experimentally infected with Trypanosoma congolense. Science World Journal 4(2).

Tomita H, Yamada O, Ohuchi H, Ono Y, Arakaki Y, Yagihara T, Echigo S (2001). Coagulation profile, hepatic function, and hemodynamics following Fontan-type operations. Cardiology in the Young 11: 62-66.

Uche UE, Jones TW (1992). Pathology of experimental *Trypanosoma evansi* infection in rabbits. Journal of Comparative Pathology 106: 299-309.

Uche UE, Jones TW (1993). Early events following challenge of rabbits with *Trypanosoma evansi* and *T. evansi* components. J. Comparative Pathology 109: 1-11.

Vigueira PA, Ray SS, Martin BA, Ligon MM, Paul KS (2012). Effects of the green tea catechin epigallocatechin-gallate on *Trypanosoma brucei*. International Journal for Parasitology. Drugs and Drug Resistance 2: 225-229.

Zargari A (1990). Medicinal Plants, (4th edn), Tehran University Publications, Tehran, pp. 14-18.

Zewdu A, Negash A, Assen A, Yaregal B (2018). Camel trypanosomosis: A review on diagnostic approaches and immunological consequences 7(3): 105-113:

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