Research Article

In Vivo Anti-Trypanosomal Activity of Basil Extract on *Trypanosoma evansi*

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Abstract | Basil is a multifunctional medicinal plant. It confirmed its anti-trypanosomal activity in vitro before. This research aimed to study its effect against *T. evansi* in vivo. For this purpose, 28 parasite-free female rats were used, seven non-infected (control group (CG)), while the others were intraperitoneally injected with *T. evansi* and then equally divided into Trypanosoma Group (TG): which remained without treatment. Diminazene aceturate group (DAG): injected with Diminazene aceturate (3.5mg/kg) at 0, 14th, and 28th days. Basil group (BG): treated with essential oil of basil (850µL/kg) at 0, 14th, and 28th days. Blood samples were collected daily for monitoring the parasitemia and the clinicopathological parameters were measured at 0 and 49 days. Tissue sections were obtained from the liver, kidney, heart, lung, spleen, and brain on the 49th day, stained, and histopathologically examined. Although, both treatments presented a significant improvement (P<0.05) in their parasitological and clinicopathological results, DAG suffered from lower levels of GR than BG, and its organs microscopical examination clarified severe lesions similar to TG. While, BG clinicopathological parameters and histopathological results were so close to CG. CK, CK-MB, LDH, and GR had high sensitivity and NPV values and moderated specificity, PPV, LR, and accuracy rate values. Conclusion: Basil plant extract is an efficient anti-trypanosomal drug. CK, CK-MB, LDH, and GR are moderate biomarkers for disease diagnosis and its treatment monitoring.

Keywords | Anti-trypanosomal activity, Basil extract, Clinicopathological alterations, Histopathological alterations, Diminazene aceturate

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INTRODUCTION

Trypanosoma evansi is a major veterinary problem that constrains camel breeding development. Its infection is usually accompanied with a significant drop in wool and milk production, abortion, infertility in males and females, and high mortalities. The treatment and prophylaxis costs are additional losses that should be taken into consideration (Mohammed et al., 2019; Darwish et al., 2019). In veterinary practice, Diminazene aceturate (DA) was always recommended for *T. evansi* treatment (Peregrine, 1994). Unfortunately, the parasite developed resistant strains against it and it also lost its effect during some stages of the disease (Witola et al., 2004). Moreover, DA has nephrotoxic and hepatotoxic effects (Spinosa et al., 1999). Hence, there is a necessity to find an alternative to it.

In the last few decades, herbal extracts attracted researchers attention. They proved their efficacy in minor and major health problems. Among them, basil which is an edible

28 apparently healthy Rattus Norvegicus (parasite-free females), weight 150-200g. They were housed in well-ventilated cages, and a sufficient amount of feed and water was allowed for them.

At 0 day of experiment

Infected groups (IG): 21 rats were intraperitoneally injected with 0.01 ml of the inoculum containing about $2x10^3$ Trypanosoma cells (the used strain was collected from naturally infected camels and identified by Barghash et al. (2016).. After the parasitemia reached $5x10^3$, the infected rats were divided as follows:

Control group (CG): 7 rats were intraperitoneally injected with 0.01 ml of normal saline.

Trypanosoma Group (TG): 7 infected rats, didn`t receive any treatment.

Diminazene aceturate group (DAG): 7 infected rats, were intraperitoneally injected with 3.5mg/kg Diminazene aceturate (trypa-ject), Adwia company® for three times: at 0, 14th, and 28th day (3rd dose).

Basil group (BG): 7 infected rats, were intraperitoneally injected with basil essential oils (850µL/kg) three times: at 0, 14th, and 28th day. Basil essential oils were execrated according to the method mentioned by (Sobhy et al., 2021).

Graph 1:

herb, is used in different cousins mainly Italian one. It was known as a general health tonic due to its rich content of antioxidants, vitamins, and minerals. In traditional medicine, it is used for snake bites, the common cold, and inflammation treatment. Recently, basil essential oils showed several therapeutic roles especially in fighting cancer, mental health support, hepatic and cardiovascular health improvement, and blood glucose levels reduction. It is also an effective anti-aging factor (Alviano et al., 2012; Aminian et al., 2022).

It has antibacterial (against *E. coli* resistant strains), anthelmintic, antiprotozoal, and antiviral activities and is used as a food preservative (Alviano et al., 2012; Kubiça et al., 2014; Sea et al., 2017; Akoto et al., 2020; Sandulachi et al., 2021). Basil displayed anti-trypanosomal characters in vitro before (Sobhy et al., 2021). Hence, this research aimed to study the *in vivo* effect of basil essential oils on *T. evansi* in experimentally infected rats and evaluate the importance of creatine kinase (CK), creatine kinase-MB (CK-MB), lactic dehydrogenase (LDH), glutathione reductase (GR) in the disease diagnosis and prognosis and its treatment follow-up.

MATERIALS AND METHODS

Animals and experiment design (Graph 1)

Parasitaemia in TG, DAG, and BG was checked daily for 49 days using wet blood film prepared from tail blood at x40 magnification. The number of parasites seen per field under the microscope was counted as described by Herbert and Lumsden (1976).

Evaluation of clinicopathological parameters

Blood samples were collected at 0 and 49 days from all rats. Each sample was separated into two portions. EDTA salt was added to the first portion to impair the coagulation process

and this portion was used immediately for hematological parameters estimation according to Tornquist (2010). The second portion was allowed to coagulate then it was centrifuged at 37°C at 3000 rpm for 20 min. The serum was separated in sterile *Eppendorf* tubes for estimation of the biochemical parameters spectrophotometrically using commercial kits of spectrum-diagnostics Egypt Company® for Biotechnology. While, serum CK, CK-MB, LDH, and GR were detected using ELISA commercial kits supplied by MyBioSource company®.

Histopathological examination

Tissue samples were collected after 49 days from the liver, kidney, heart, lung, spleen and brain of all animals, then fixed in 10% neutral buffered formal-saline for preparing paraffin tissue sections. These sections were stained with hematoxylin and eosin (Bancroft and Gamble, 2002).

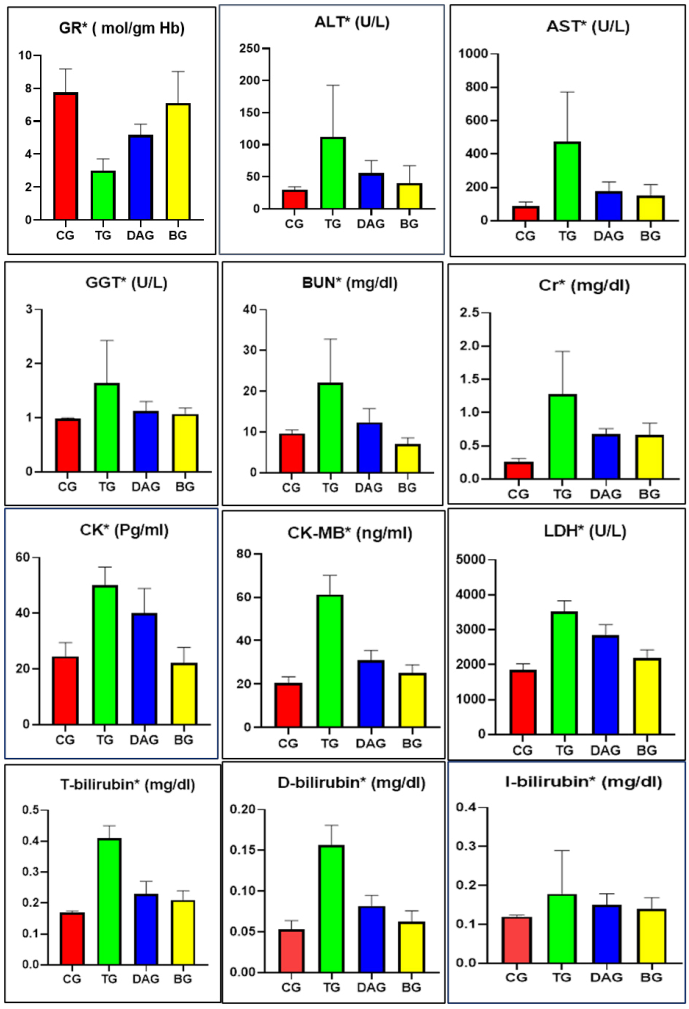

Figure 1: Comparison of oxidative stress and organs function tests between the studied groups, differences between groups were considered significant when P < 0.05 and indicated by (*).

Statistical analysis

Data were analyzed using SPSS version 20.0 (IBM SPSS Statics 20, USA), using one way-ANOVA test to compare between means of different statistical parameters. Values of P<0.05 were regarded as statistically significant.

Graph Pad Prism version 8 program was used to calculate the cut-off points, sensitivity, specificity, and likelihood ratio (LR) for CK, CK-MB, LDH, and GR in TG compared to CG and in treated groups (DAG, BG (TGs)) compared to TG.

The positive predictive value (PPV), negative predictive value (NPV), and accuracy rate for them is the result of dividing the number of true positive or true negative or the sum of both on the number of the total positive or total negative or total population respectively, then multiplied in 100.

The percentage of increase or decrease for each one of them was calculated following the next equation:

Mean value of marker conc. in (TG,DAG, BG) - Mean value of marker conc. in (CG, TG, TG) ÷ Mean value of marker conc, in (TG, DAG, BG) × 100

RESULT AND DISCUSSION

T. evansi is a real threat to camel breeding. Its infection usually stimulates the host immune system to release pro-inflammatory cytokines, which induce other immune cells to pose several anti-parasitic immune products. Among them are free radicals, which destroy the invading parasite through their vital component oxidation. Anti-oxidant enzymes and vitamins are responsible for their neutralization to protect the host cells from their harmful effect (Baldissera et al., 2016; El-Bahr and El-Deeb, 2016). Unfortunately, prolonged exposure to the pathogen induces free radicals massive production and accumulation causing antioxidants consumption and depletion. These free radicals attack the host cells and destruct them, leading to oxidative stress appearance. On the same harmony, the current study showed a significant (P<0.05) decrease in GR levels in TG (in relation to CG) accompanied by a significant elevation of hepatic enzymes activities (ALT, AST, GGT), renal function tests (BUN, Cr), CK (skeletal muscle damage indicator), CK-MB (heart damage indicator), and LDH (lung damage indicator) in TG (when compared to CG) (Figure 1). Similar observations were recorded in *T. evansi* infection by Darwish et al. (2019) and Mohammed et al. (2019). In parallel, The histopathological results of TG described a liver with congestion, area of coagulative necrosis, inflammatory cell infiltration and vacuolar degeneration of hepatocytes (Figure 4), congested kidney with tubular necrosis and increase space of Bowman's capsule (Figure 5), heart with large area of hemorrhage, edema and myocardiolysis necrosis of muscle (Figure 6), lung with bronchitis and bronchiolitis, peribronchial edema, hyperplasia

of epithelial lining and sever peribronchial infiltration of chronic inflammatory cells and alveolar emphysema (Figure 7), spleen with hemorrhage and congestion, vascular and perivascular edema and depletion of white pulp (Figure 8), brain with demyelination and neuronal chromatolysis and edema and perivascular edema (Figure 9). In addition to the oxidative, the activated pro-inflammatory cytokines enhance the vasodilators production, which increase the vascular permeability causing the inflammatory cells infiltration in different organs and lymphocytes deposition in the lymph nodes and spleen causing their enlargement (Darwish et al., 2019; Mohammed et al., 2019).

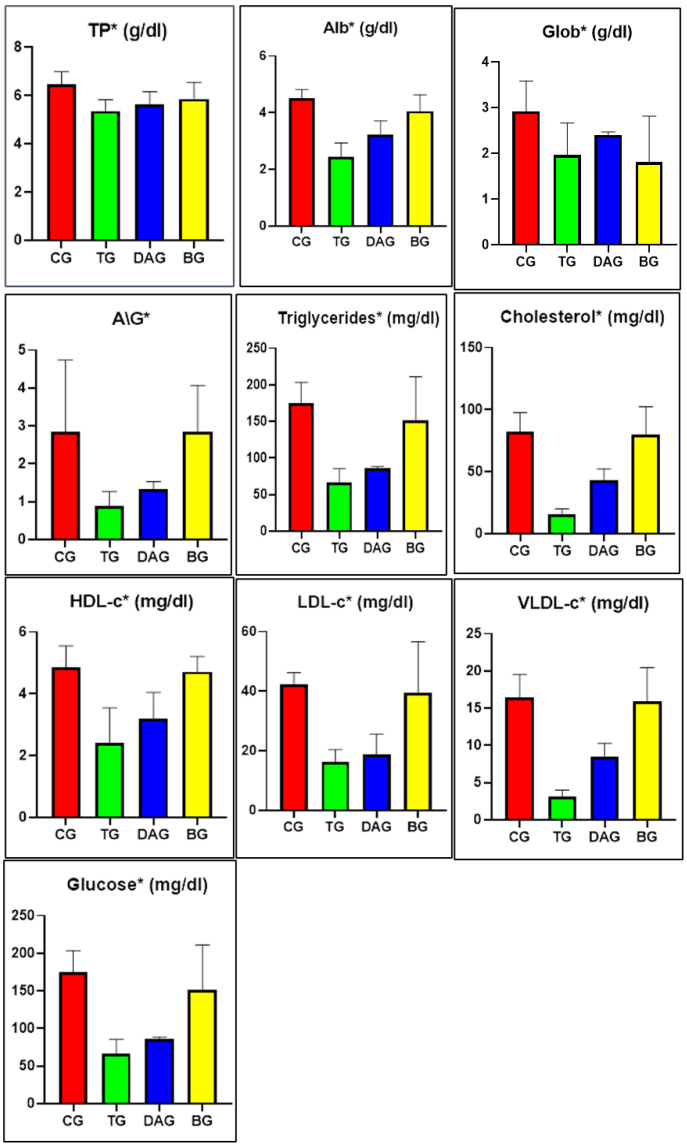

Figure 2: Comparison of glucose, protein, and lipid profiles between the studied groups, differences between groups were considered significant when P < 0.05, and indicated by (*).

The oxidative stress also has a major involvement in the significant (P<0.05) hyperbilirubinemia (T/I/D) noticed in TG (when compared to CG) (Figure 1). As, free radicals destroy the RBCs membrane leading to I-bilirubin leakage in the circulation and the hepatocytes oxidative damage prevents I-bilirubin clearance from circulation and impairs D-bilirubin excretion through the liver (Mbaya et al., 2014; Gopalakrishnan et al., 2019). In the same way, the significant (P<0.05) hypoproteinemia (hypoalbuminemia and hypglobulinemia), hypocholesterolemia (T/HDL/LDL/VLDL), and hypoglycemia obtained in TG (in relation to CG) were attributed to the liver oxidative damage (Figure 2). As the liver is the major organ responsible for albumin, globulin (α, β), and cholesterol synthesis, and blood glucose levels regulation (via glycogenolysis and gluconeogenesis).

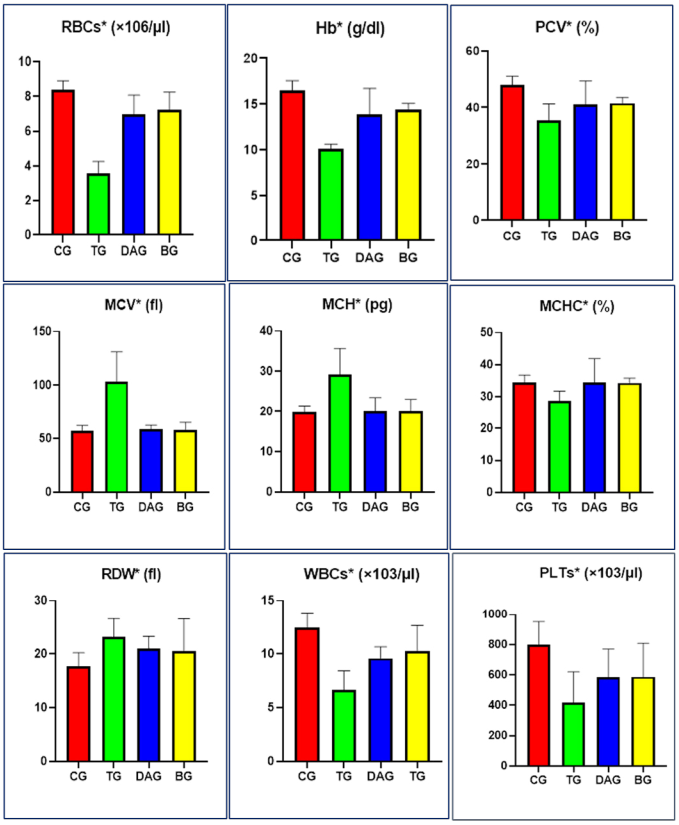

Figure 3: Comparison of hematological parameters between the studied groups, differences between groups considered significant when P < 0.05 and indicated by (*).

So, its damage means lower levels of blood protein, cholesterol, and glucose (Sivajothi et al., 2015; Eze et al., 2015; Darwish et al., 2019). Additionally, the anti-oxidant characters of albumin and HDL-cholesterol make them a target for the circulating free radicals, therefore the oxidative stress participated in the outstanding hypoproteinemia and hypocholesterolemia in TG through another way (Mbaya et al., 2014; Sivajothi et al., 2015; Darwish et al., 2019). Interestingly, the cytokines-induced pyrexia and anorexia and subsequent amino acids, free fatty acids, and glucose deficiency took part in the noted hypoproteinemia, hypocholesterolemia, hypotriglyceridemia, and hypoglycemia in TG (Mbaya et al., 2014; Sivajothi et al., 2015; Darwish et al., 2019).

Furthermore, Trypanosoma massively absorbs the host lipids and glucose due to its opportunistic nature and its inability to synthesize the lipids and glucose necessary for its survival and multiplication (Mbaya et al., 2014; Sivajothi et al., 2015; Darwish et al., 2019).

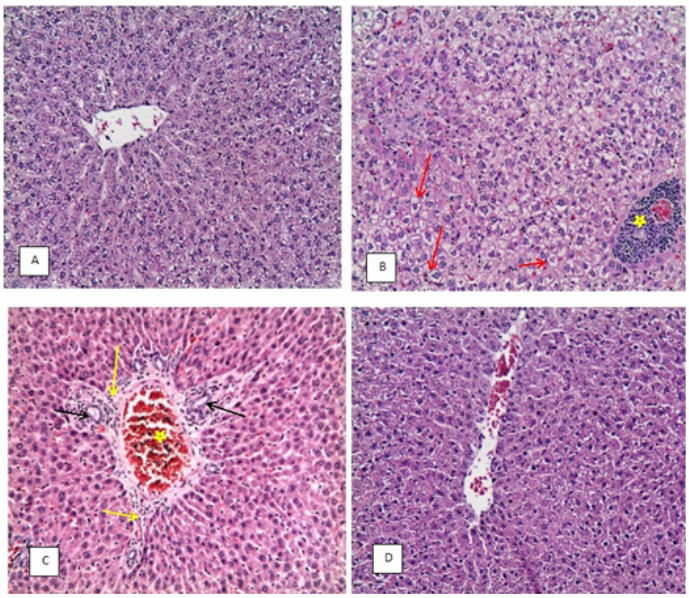

Figure 4: (H&E X20). **A:** liver of CG, **B:** liver of TG showing congestion, area of coagulative necrosis with inflammatory cell infiltration (star) and vacuolar degeneration of hepatocytes (red arrow), **C:** liver of DAG showing congestion and edema (star), infiltration of inflammatory cells (yellow arrow), newly formed bile duct (black arrow) and dilatation of hepatic sinusoids, **D:** liver of BG more or less to normal with slight congestion.

T. evansi also impairs the host hematopoiesis, resulting in the macrocytic hypochromic anemia noticed in TG in the current work. This anemia was indicated by the significant (P<0.05) decrease in RBCs, Hb, PCV, MCH, MCHC in TG in relation to CG and the significant (P<0.05) increase in MCV in TG in relation to CG) (Figure 3). Previous studies attributed this anemia to the mechanical hemolysis of RBCs because of the Trypanosoma presence and movement in the host bloodstream. Others assigned this anemia to the oxidative damage of RBCs due to the aforementioned oxidative stress (Ahmadi-hamedani et al., 2014; Darwish et al., 2019). In addition, the trypanosomal enzymes inhibit the erythropoiesis and hydrolyze the RBCs membrane (Mohammed et al., 2019). With the disease progression, the bone marrow starts releasing reticulocytes and immature RBCs in the host circulation. Consequently, the anemia become macrocytic hypochromic anemia and MCV and RDW values significantly (P<0.05) increased (Zewdu et al., 2016; Darwish et al., 2019). This response may cause bone marrow exhaustion leading to the significant (P<0.05) leukopenia and thrombocytopenia observed in TG when compared to CG (Figure 3) (Tribulatti et al., 2005; Adeyeye et al., 2017). The trypanosomal sialidase enzyme, lymphocytes migration to the affected organs (due to inflammatory reaction), and eosinophilic trapping in the spleen are other possible reasons for the spotted leukopenia and thrombocytopenia here (Adeyeye et al., 2017). These biochemical and hematological alteration and heavy protozoal infestation (noted in the microscopical examination of TG blood smears) explained the intense clinical signs appeared on the infected animals as emaciation, in appetence, pyrexia, dullness and corrugated skin at 49 days.

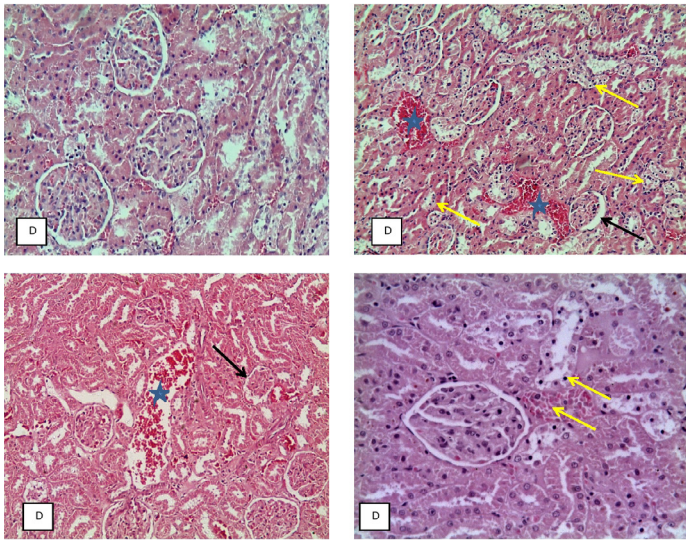

Figure 5: (H&E X20), **A:** kidney of CG, **B:** kidney of TG showing congestion (star), tubular necrosis (yellow arrow) and increase space of Bowman's capsule (black arrow), **C:** kidney of DAG showing congestion and edema (arrow), necrosis of tubules and atrophy of glomeruli (black arrow), **D:** kidney of BG more or less to normal with slight congestion and necrosis of tubules (yellow arrow).

After treatment

Both treatments successfully removed the parasite and its lethal products and counteracted the oxidative stress connected to the disease. Therefore, the animals restored their appetite, and their clinicopathological parameters levels significantly (P<0.05) enhanced, approaching CG levels. These findings mimicked previous studies that mentioned DA inhibitory effect on trypanosomal DNA replication. As it binds to its kinetoplast DNA (kDNA) in a non-intercalative way via specific interaction with sites rich in adenine-thymine base pairs (Peregrine, 1994). Besides, its anti-inflammatory antioxidant action which mediated through pro-inflammatory cytokines suppression. Therefore, it partially reverses the clinicopathological and histopathological alterations encountered with the disease (Kuriakose et al., 2012, 2014). Similarly, the basil essential oils approved its potent anti-trypanosomal activity *in vitro* before. They easily prohibit trypanosomal growth within 60 minutes, by interfering with the protozoal cell membrane permeability and reacting with its proteins and

lipids (Borges et al., 2012; Sobhy et al., 2021). In addition, basil essential oils have anti-inflammatory, antioxidant, and anti-microbial properties (Borges et al., 2012; Sobhy et al., 2021).

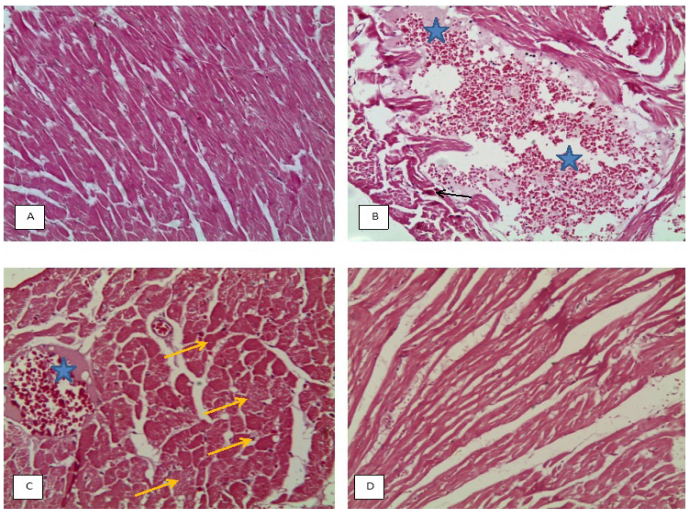

Figure 6: (H&E X20), **A:** heart of CG, **B:** heart of TG showing large area of hemorrhage and edema, myocardiolysis (star) and necrosis of muscle (black arrow), **C:** heart of DAG showing myocardiolysis, hemorrhage and edema (star), swelling of muscles and muscle necrosis (yellow arrow), **D:** heart of BG more or less to normal with slight myocardiolysis.

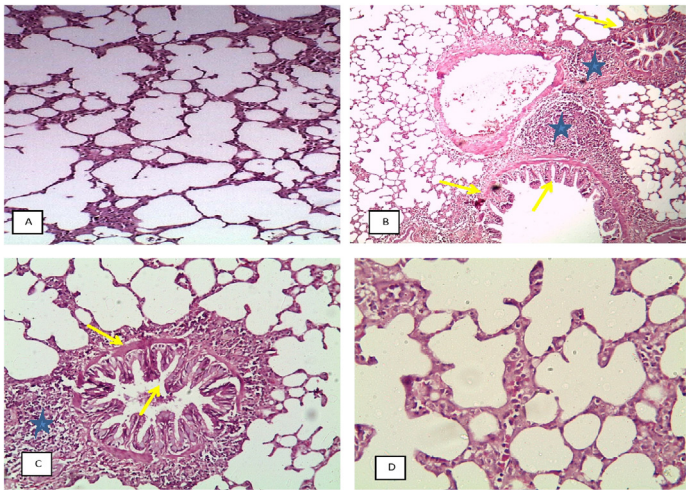

Figure 7: (H&E X4), **A:** lung of CG showing alveolar emphysema, **B:** lung of TG showing bronchitis and bronchiolitis, peribronchial edema, hyperplasia of epithelial lining (yellow arrow) and sever peribronchial infiltration of chronic inflammatory cells (star) and alveolar emphysema, **C:** lung of DAG showing catarrhal bronchiolitis, hyperplasia of epithelial lining, edema (yellow arrow) with massive infiltration of chronic inflammatory cells, (star) and alveolar emphysema, **GD:** lung of BG beginning to return to normal showing alveolar emphysema.

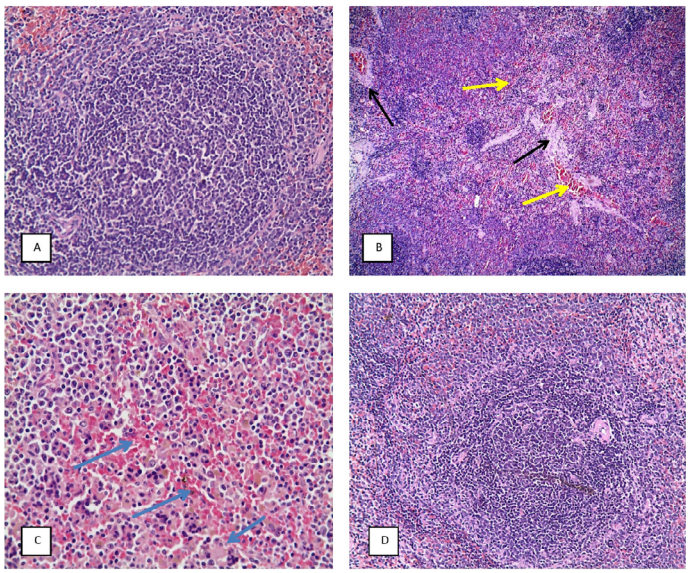

Figure 8: (H&E X20), **A:** spleen of CG, **B:** spleen of TG showing splenic hemorrhage and congestion (yellow arrow), vascular and perivascular edema (black arrow) and depletion of white pulp, **C:** spleen of DAG showing splenic hemorrhage (blue arrow), edema and depletion of white pulp, **D:** spleen of BG showing more or less to normal.

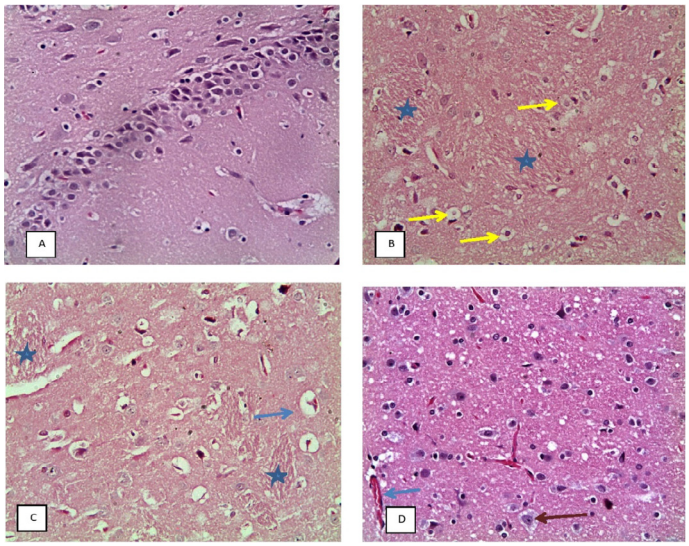

Figure 9: (H&E X20), **A:** showing CG brain, **B:** brain of TG showing demyelination (star), chromatolysis of neuron, neuronal edema (yellow arrow) and perivascular edema. **C:** brain of DAG showing demyelination of brain (star) and perivascular edema (blue arrow), **D:** brain of BG showing decreased lesion severity, chromatolysis of neuron (red arrow) and congested blood vessels (blue arrow).

The comparison between the two drugs

At the 49th day, basil extract was more effective than DA in the trypanosomiasis consequences curing and the host body protection. Although BG showed a slight degree of thrombocytopenia and elevated Cr levels at the end of the experiment (when compared to CG), most of its group hematological and biochemical parameters returned to its

Table 1: Cut-off points, sensitivity %, specificity %, likelihood ratios (LR), PPV%, NPV%, and accuracy rate% in TG (in relation to CG) and TGs (DAG+ BG in relation to TG).

Statistical parameters	CK (Pg/ml)		CK-MB (ng/ml)		LDH(U/L)		GR (mol/gm Hb)	
	TG	TGs	TG	TGs	TG	TGs	TG	TGs
Cut-off points	27.05	49.51	21.97	56.92	1938	3397	7.04	3.36
Sensitivity	100%	100%	100%	100%	100%	100%	100%	100%
Specificity	66.67%	66.67%	66.67%	66.67%	66.67%	66.67%	66.67%	66.67%
LR	3	3	3	3	3	3	3	3
PPV	75%	85.71%	75%	85.71%	75%	85.71%	75%	85.71%
NPV	100%	100%	100%	100%	100%	100%	100%	100%
Accuracy rate	88.89%	88.89%	88.89%	88.89%	88.89%	88.89%	88.89%	88.89%

normal ranges (Figures 1-3) , and organs microscopical examination was so close to those of CG (Figures 4,5,6,7,8 and 9). This result returned to basil-rich content of essential oils, mainly linalool and 1, 8-cineole, which prohibit the cytokines storm accompanied the disease course, and the dependent oxidative stress (Borges et al., 2012; Sobhy et al., 2021). In contrast, DAG had a pronounced degree of oxidative stress by the end of the study, indicated by the low GR levels and dependent high liver and kidney function tests, CK, CK-MB and LDH noticed in DAG (when compared to CG and BG) (Figures 1-3) (Spinosa et al., 1999; Sobhy et al., 2021). The histopathological results of DAG were concomitant with these results, it depicted marked degenerative lesions with hemorrhage, edema and cellular infiltration in the examined organs (Figures 4, 5, 6, 7, 8 and 9). The liver damage is an acceptable reason for the hypoalbuminemia (connected hypoproteinemia and decreased A/G), hypocholesteremia, and hypoglycemia reported in DAG in relation to CG, BG (Figure 2) as illustrated before in this work (Sivajothi et al., 2015; Eze et al., 2015; Darwish et al., 2019). Meanwhile, the diminished RBCs, WBCs, and PLTs values in DAG pointed to the absence of the bone marrow regenerative response due to its fatigue (Figure 3) (Adeyeye et al., 2017).

The value of CK, CK-MB, LDH, and GR in trypanosomiasis diagnosis and prognosis and following-up its treatment: They were moderately effective markers for trypanosomiasis as they yielded sensitivity and NPV AS 100% and moderate values of specificity, accuracy rate, and low likelihood ratios (Table 1). While the percentage of increase (or decrease) nominated CK-MB as a disease marker and GR for its treatment monitoring (Table 2).

Table 2: Percentages of increase (+) or decrease (-) in TG (in relation to CG), DAG (in relation to TG), BG (in relation to TG).

	CK	CK-MB	LDH	GR
TG	103.58%	197.09%	90.22%	-61.11%
DAG	-19.81%	-49.77%	-19.51%	71.43%
BG	-55.49%	-59.41%	-38.18%	135.88%

CONCLUSION

Basil plant extract has is more effective than DA for treatment of *T. evansi* infection. Among the estimated markers, CK-MB is the best for the trypanosomiasis diagnosis and GR is the best for its treatment evaluation. For more accuracy, further field studies on camels should be applied.

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NOVELTY STATEMENT

The research recommended basil extract as a new treatment for Trypanosoma evansi infection and suggested CK-MB for its diagnosis and GR for its treatment evaluation.

AUTHOR'S CONTRIBUTION

All authors contributed equally.

Conflict of interest

The authors have declared no conflict of interest.

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