Journal of Current Veterinary Research

ISSN: 2636-4026

Journal homepage: http://www.jcvr.journals.ekb.eg

Internal Medicine & Infectious Disease

The Effect of Chamomile Extract on *Rattus Norvegicus* Infected with *Trypanosoma evansi*

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ABSTRACT

Chamomile is a multi-purpose medicinal plant. It approved its anti-trypanosomal effect *in vitro* before. This work aimed to study its anti-trypanosomal effect *in vivo*. For this aim, 28 parasite-free rats were used, seven remained non-infected (control group (CG)), while the others were infected with *Trypanosoma evansi* and then equally divided into *Trypanosoma* Group (TG): didn`t receive any treatment. Diminazene aceturate group (DAG): treated with Diminazene aceturate (3.5 mg/kg) on days 0, 14, and 28.Chamomile group (CAG): treated with chamomile extract (1300 µl/kg) on days 0, 14, and 28. Blood samples were collected daily for monitoring the parasitemia and the clinicopathological parameters were estimated on days 0 and 49. Tissue sections were collected from the liver, kidney, heart, lung, spleen, and brain on day 49, stained, and histopathologically examined. Although, both treatments displayed a significant ($P<0.05$) amelioration of the parasitological and clinicopathological results. DAG suffered from a higher degree of oxidative stress than CAG and the microscopical examination of their organs showed intensive lesions similar to TG. While CAG clinicopathological parameters and histopathological results approximately were similar to CG. Creatine kinase, creatine kinase-MB, lactic dehydrogenase, and glutathione reductase had high sensitivity and negative predictive value, and moderate specificity, positive predictive value, and accuracy rate. Conclusion: chamomile extract is an effective anti-trypanosomal drug. Creatine kinase, creatine kinase-MB, lactic dehydrogenase, and glutathione reductase are moderate markers for the disease diagnosis and its treatment evaluation.

Keywords: Anti-trypanosomal activity, Chamomile extract, Clinicopathological alterations, Diminazene aceturate and Histopathological alterations.

INTRODUCTION

Trypanosomiasis is a protozoal infection, which causes severe damage to different body organs. *Trypanosoma* has several species so it targets a wide section of wild and domestic animals, birds even humans (Mohammed et al., 2019; Darwish et al., 2019). Among its dangerous species, is *Trypanosoma evansi* which basically attacks dromedaries, reducing their milk and meat production. It also interferes with their immune system function and increases their susceptibility to other infectious diseases. Sterility, abortion, therapeutic interventions cost, and sudden death are other economic losses associated with the disease (Mohammed et al., 2019; Darwish et al., 2019).

In the veterinary field, Diminazene aceturate (DA) is the first drug choice for

Trypanosoma treatment (Peregrine, 1994). Although its efficacy and low price, it has pronounced toxic effects on the kidneys and liver (Spinosa et al., 1999). In addition, DA failed in controlling the parasitemia related to the disease as some *T. evansi* strains developed resistance (Witola et al., 2004). Therefore, there is a persistent need to find a substitute for DA.

Since the time of Hippocrates, different herbal extracts used in the treatment of major and minor health problems. *Matricaria chamomilla* (Chamomile) extract is one of the most important herbal extracts. It is widely used in human medicine (Srivastava et al., 2010; Zafari Zangeneh et al., 2010), as it contains more than 120 pharmacologically active chemical constituents. They include chamazulene (anti-inflammatory), bisabolol (anti-irritant, anti-inflammatory, anti-microbial), apigenin (a phytonutrient that acts as a strong anti-inflammatory, anti-oxidant, anti-protozoal, antibacterial and antiviral) and luteolin (a phytonutrient with potential anti-oxidant, anti-protozoal, anti-inflammatory and anti-cancer activity) (AbouLaila et al., 2018; AbouLaila et al., 2019; Elazab et al., 2020; Sobhy et al., 2021). Recently, Chamomile extract confirmed its anti-parasitic and anti-trypanosomal action *in vitro* (Sobhy et al., 2021; El Mihyaoui et al., 2022).

Hence, this research aimed to study the trypanocidal activity of Chamomile extract on *Trypanosoma evansi* infected rats, with special reference to the value of creatine kinase (CK), creatine kinase-MB (CK-MB), lactic dehydrogenase (LDH), and glutathione reductase (GR) in *T. evansi* diagnosis and prognosis and tracking of its treatment.

MATERIAL AND METHODS

Animals and experiment:

After the ethical approval of the animal and poultry health department, Desert Research Centre, Cairo, Egypt, 28 healthy *Rattus Norvegicus* (parasite-free) female rats (150-200g) were kept in well-ventilated plastic cages, adequately balanced ration and sufficient amount of water were introduced for them. After their adaptation and acclimatization, (on day 0) seven rats were intraperitoneally injected with 0.01 ml of normal saline (control group (CG)). The other 21 were intraperitoneally injected with 0.01 ml of the inoculums containing about $2x10^3$Trypanosoma cells (the used strain was collected from naturally infected camels and identified by Barghash et al. (2016). Parasitemia was monitored by microscopical examination till reaching $5x10^3$, then divided as follows: *Trypanosoma* Group (TG): 7 infected rats, remained without treatment. Diminazene aceturate group (DAG): 7 infected rats, were intraperitoneally injected with 3.5 mg/kg Diminazene aceturate, Adwia company® three times: 1st dose on day 0, 2nd dose on day 14, 3rd dose on day 28. The drug dose and route of administration were recommended by the manufacturing company. Chamomile group (CAG):7 infected rats, were intraperitoneally injected with chamomile extract (1300 µl/kg) three times: 1st dose on day 0, 2nd dose on day 14, 3rd dose on day 28. Chamomile extract was prepared according to the method described by Sobhy et al. (2021).

Parasitemia in TG, DAG, and CAG was checked daily for 49 days using the wet blood film technique. The number of parasites seen per field was counted by Herbert and Lumsden (1976) method.

Evaluation of clinicopathological parameters:

Blood samples were collected on days 0 and 49 from all rats. Each sample was separated into two portions. EDTA salt was added to the first portion to stop the coagulation process and this portion was used immediately for the evaluation of the hematological parameters according to Tornquist (2010). The second portion was allowed to coagulate then it was centrifuged at 37 ºC 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 My BioSource company®, San Diego, USA.

Histopathological examination:

Tissue samples were collected on day 49 from all animals' brains, hearts, lungs, livers, spleens, and kidneys, 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).

Statistical analysis:

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

GraphPad Prism version 8 program was used to calculate the cut-off points, sensitivity, specificity, and likelihood ratio (LR) for CK, CK-MB, LD, and GR in TG compared to CG and in treated groups (DAG, CAG (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 total positive or the 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:

$$\text{mean value of marker conc. in (TG, DAG, CAG)} - \text{mean value of marker conc. in (CG, TG, TG)} \div \text{mean value of marker conc. in (TG, DAG, CAG)} * 100$$

RESULTS

TG suffered from anorexia, dullness, depression, rough skin, weakness, and emaciation. These signs intensity increased throughout the experiment, reaching their maximum level on day 49. The parasitological examination of the blood samples revealed a heavy protozoal infection. In accordance, the biochemical parameters of TG (when compared to CG) cleared a significant ($P<0.05$) decline in GR, TP, Alb, Glob, triglycerides, T/HDL/LDL/VLDL- cholesterol, glucose, and a significant ($P<0.05$) increment in the liver function tests (ALT, AST, GGT, and T/D/I-bilirubin) and renal function tests (BUN, Cr), CK, CK-MB, and LDH (Table 1). The hematological examination of TG illustrated a macrocytic hypochromic anemia accompanied with leukopenia and thrombocytopenia (displayed by the significant ($P<0.05$) decrease in RBCs, Hb, PCV, MCH, MCHC, WBCs, and PLTs in TG in relation to CG and the significant ($P<0.05$) increase in MCV and RDW in TG in relation to CG (Table 2)).The histopathological results of TG depicted congested liver with an area of coagulative necrosis and vacuolar degeneration of hepatocytes (Fig. 1). Kidney with edema and hemorrhage, tubular necrosis, and appearance of cast inside the tubules, and slight fibrosis (Fig. 2).Heart with myocardiolysis, edema, muscle necrosis, hemorrhage, and inflammatory cells infiltration (Fig. 3).Lung with bronchitis, peribronchial edema and massive infiltration of chronic inflammatory cells, lymphocytic granuloma, and compensatory emphysema (Fig. 4). Spleen with hemorrhage, vascular and perivascular edema, and depletion of white pulp (Fig. 5). Brain with demyelination, perivascular edema and chromatolysis of neuron, and neuronal edema (Fig. 6).

After treatment:

Both treated groups (DAG and CAG) showed a gradual regression of the clinical signs and the parasitological examination of their blood films showed were negative

on day 7 then *T. evansi* reappeared again in small numbers and increased gradually till day 14 then disappeared after the second dose of DA or Chamomile extract then reappeared and reached its peak on day 28 but after the third dose it completely vanished and the blood samples were negative on day 49. In parallel, the clinicopathological parameters levels of both groups (when compared to TG) significantly ($P<0.05$) increased or decreased towards CG parameters levels.

On day 49, DAG (when compared to CG and CAG) exposed a significant ($P<0.05$) elevation in the liver function tests (ALT, AST, and T/D-bilirubin) and renal function tests (Cr), CK, CK-MB, and LDH and a significant ($P<0.05$) reduction in GR, TP, Alb, A/G, triglycerides, T/HDL/LDL/VLDL- cholesterol, glucose, RBCs, WBCs, and PLTs levels (Table 1, 2). The histopathological examination of DAG organs showed lesions similar to those of TG (Fig. 1-6).

On the other hand, CAG values were better than DAG values and more close to CG values and most of them non-significantly ($P\geq0.05$) changed with CG (except GR, TP, and PLTs significantly ($P<0.05$) decreased and CK-MB significantly ($P<0.05$) increased in relation to CG) (Table 1,2). The histopathological examination of CAG described approximately normal liver, kidney, heart, and spleen while the alveolar emphysema and hemorrhage still present in the lung and the brain revealed neural and perivascular edema (Fig. 1-6).

Regarding the value of CK, CK-MB, LDH, and GR in trypanosomiasis diagnosis and prognosis, and following-up its treatment, Table (3) clarified that all of them yielded sensitivity and NPV as 100% and moderate values of specificity, PPV, accuracy rate, and low likelihood ratios. While the percentage of increase (or decrease) nominated CK-MB for trypanosomiasis detection and GR for its treatment monitoring (Table 4).

Table (1): Comparison between the biochemical parameters of the studied groups:

Parameters	CG	TG	DAG	CAG
GR(mol/gm Hb)	7.75 ± 0.54^{d}	3.00 ± 0.31^{a}	5.16 ± 0.29^{ab}	6.28 ± 0.48^{abc}
ALT (U/L)	30.00 ± 1.70^{d}	112.40 ± 9.67^{a}	55.80 ± 8.78^{ab}	20.83 ± 5.82^{bc}
AST (U/L)	89.57 ± 8.91^{d}	474.00 ± 39.65^{a}	174.60 ± 10.40^{ab}	59.00 ± 9.09^{bc}
GGT(U/L)	1.00 ± 0.00^{d}	1.64 ± 0.31^{a}	1.12 ± 0.08^{b}	1.06 ± 0.13^{b}
T-bilirubin (mg/dl)	0.17 ± 0.005^{d}	0.41 ± 0.016^{ab}	0.23 ± 0.018^{ab}	0.19 ± 0.021^{b}
D-bilirubin (mg/dl)	0.05 ± 0.004^{d}	0.15 ± 0.014^{a}	0.08 ± 0.005^{ab}	0.068 ± 0.004^{b}
I-bilirubin (mg/dl)	0.12 ± 0.004^{d}	0.25 ± 0.016^{a}	0.15 ± 0.013^{b}	0.12 ± 0.02^{b}
BUN(mg/dl)	9.67 ± 0.31^{d}	22.05 ± 0.01^{ab}	12.43 ± 1.46^{b}	8.11 ± 1.37^{b}
Cr(mg/dl)	0.25 ± 0.02^{d}	1.28 ± 0.28^{a}	0.68 ± 0.03^{ab}	0.30 ± 0.05^{bc}
CK (Pg/ml)	24.60 ± 1.84^{d}	50.08 ± 2.92^{a}	40.16 ± 3.92^{ab}	22.80 ± 1.28^{bc}
CK-MB(ng/ml)	20.64 ± 1.00^{d}	61.32 ± 3.94^{a}	30.80 ± 2.08^{ab}	28.61 ± 2.00^{ab}
LDH(U/L)	1855 ± 63.05^{d}	3528.6 ± 133.88^{a}	2839.4 ± 136.2^{ab}	1953.66 ± 70.199^{bc}
TP(g/dl)	6.74 ± 0.13^{d}	4.08 ± 0.04^{a}	5.24 ± 0.25^{ab}	5.98 ± 0.03^{abc}
Alb(g/dl)	4.50 ± 0.12^{d}	2.44 ± 0.22^{a}	3.22 ± 0.22^{ab}	4.04 ± 0.29^{bc}
Glob (g/dl)	2.24 ± 0.15^{d}	1.64±0.24a	2.02±0.05	1.94±0.29
A\G	2.07 ± 0.17^{d}	1.90±0.07	1.59 ± 0.09^{ab}	1.73±0.03
Triglycerides (mg/dl)	82.14 ± 5.81^{d}	15.50 ± 2.00^{a}	43.00 ± 4.11^{ab}	96.00 ± 12.07^{bc}

Cholesterol (mg/dl)	63.57±1.95[d]	21.80±2.71[a]	30.60±3.37[ab]	62.33±2.10[bc]
HDL-c (mg/dl)	4.85±1.95[d]	2.40±0.50[a]	3.20±0.37[a]	5.33±0.21[bc]
LDL-c (mg/dl)	42.28±1.51[d]	16.30±1.84[a]	18.76±3.08[a]	37.80±2.11[bc]
VLDL-c (mg/dl)	16.42±1.16[d]	3.10±0.40[a]	8.64±0.82[ab]	19.20±2.41[bc]
Glucose (mg/dl)	174.57±22.37[d]	66.20±8.72[a]	86.60±4.85[a]	169.67±21.17[bc]

Values are mean± SD.[a] (significant with CG), [b] (significant with TG), [c] (significant with DAG), [d] (significant among the four studied groups), considered significant when P<0.05.
GR: glutathione reductase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamyl transferase, T/D/I-bilirubin: total/direct/indirect-bilirubin, BUN: blood urea, Cr: creatinine, CK: creatinine, CK: creatine kinase, CK-MB: creatine kinase-MB, LDH: lactic dehydrogenase, TP: total protein, Alb: Albumin, Glob: globulin, A/G: albumin/globulin, HDL/LDL/VLDL-c: high density /low density/very low density lipoprotein-cholesterol.

Table (2): Comparison between the hematological parameters of the studied groups:

Parameter	CG	TG	DAG	CAG
RBCs ($\times10^6$/µl)	8.37±0.20[d]	3.65±0.31[a]	6.99±0.49[ab]	7.40±0.55[b]
Hb (g/dl)	16.51±0.39[d]	10.06±0.25[a]	13.87±1.27[ab]	14.47±0.60[b]
PCV (%)	47.92±1.18[d]	35.38±2.65[a]	41.02±3.72	43.86±3.36[b]
MCV (fl)	57.38±1.80[d]	103.12±12.50[a]	58.42±1.86[b]	59.70±3.75[b]
MCH (pg)	19.77±0.62[d]	29.26±2.89[a]	19.93±1.56[b]	20.04±1.17[b]
MCHC (%)	34.52±0.85[d]	28.81±1.33[a]	34.47±3.32[b]	33.85±1.85
RDW (fl)	17.70±0.95[d]	23.23±1.50[a]	20.94±1.05	17.91±1.38[b]
WBCs ($\times$103/µl)	12.45±0.52[d]	6.68±0.78[a]	9.60±0.47[ab]	11.12±1.48[ab]
PLTs ($\times10^3$/µl)	800.71±57.17[d]	415.40±91.65[a]	585.10±82.52[a]	581.83±74.89[a]

Values are mean± SD. [a] (significant with CG), [b] (significant with TG), [c] (significant with DAG), [d] (significant among the four studied groups), considered significant when P<0.05.
RBCs: red blood cell count, Hb: hemoglobin concentration, PCV: packed cell volume, MCV: mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration, RDW: red cell distribution width, WBCs: white blood cell count, PLTs: platelets count.

Table (3): Cut-off points, sensitivity%, specificity%, Likelihood Ratios (LR), PPV%, NPV%, accuracy rate% in TG (in relation to CG), and TGs (DAG+ CAG in relation to TG):

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	**25.58**	**48.20**	**21.14**	**59.35**	**1887**	**3461**	**7.48**	**3.156**

points								
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	**88.89 %**	**88.89 %**	**88.89 %**	**88.89 %**	**88.89 %**	**88.89 %**	**88.89 %**	**88.89 %**

Table (4): Percentages of increase (+) or decrease (-) in TG (in relation to CG), DAG (in relation to TG), and CAG (in relation to TG):

	CK	CK-MB	LDH	GR
TG	103.58%	197.09%	90.22%	-61.29%
DAG	-24.70%	-49.77%	-19.53%	72%
CAG	-54.47%	-53.34%	-44.63%	109.33%

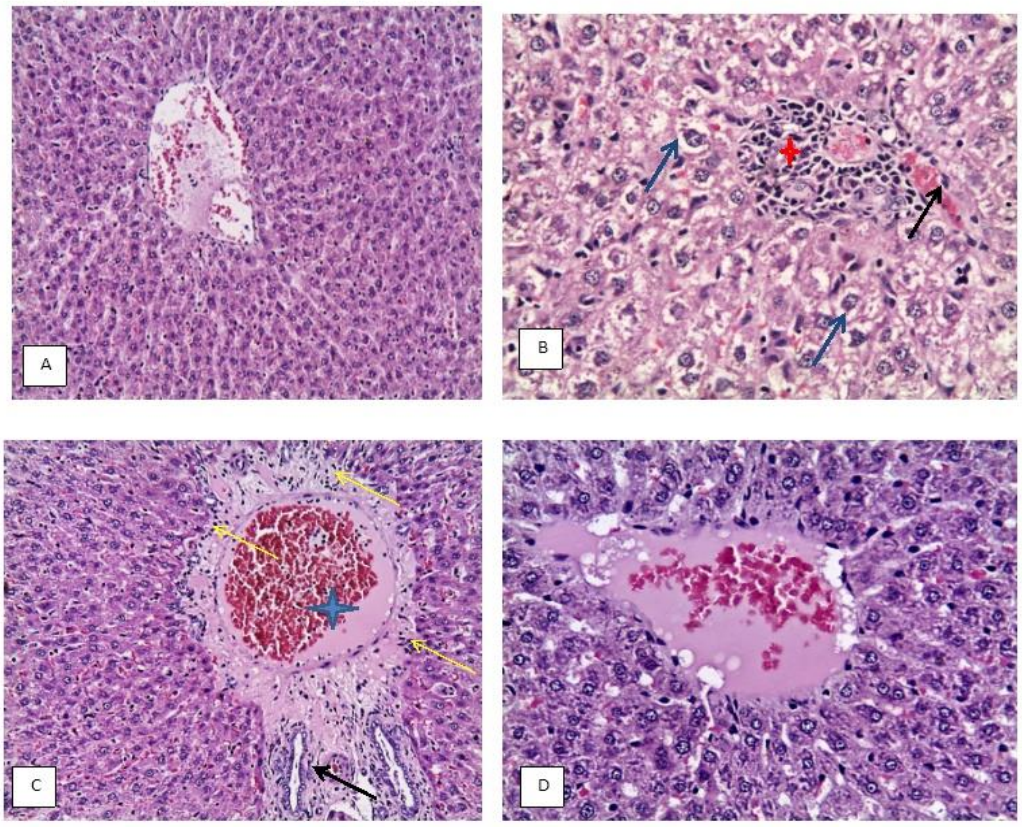

Figure 1:

A: liver of CG.

B: liver of TG showing congestion (arrow), area of coagulative necrosis (star), and vacuolar degeneration of hepatocytes (yellow arrow).

C: liver of DAG showing congestion and edema (star), infiltration of inflammatory cells (yellow arrow), and newly formed bile duct (black arrow).

D: liver of CAG more or less to normal.

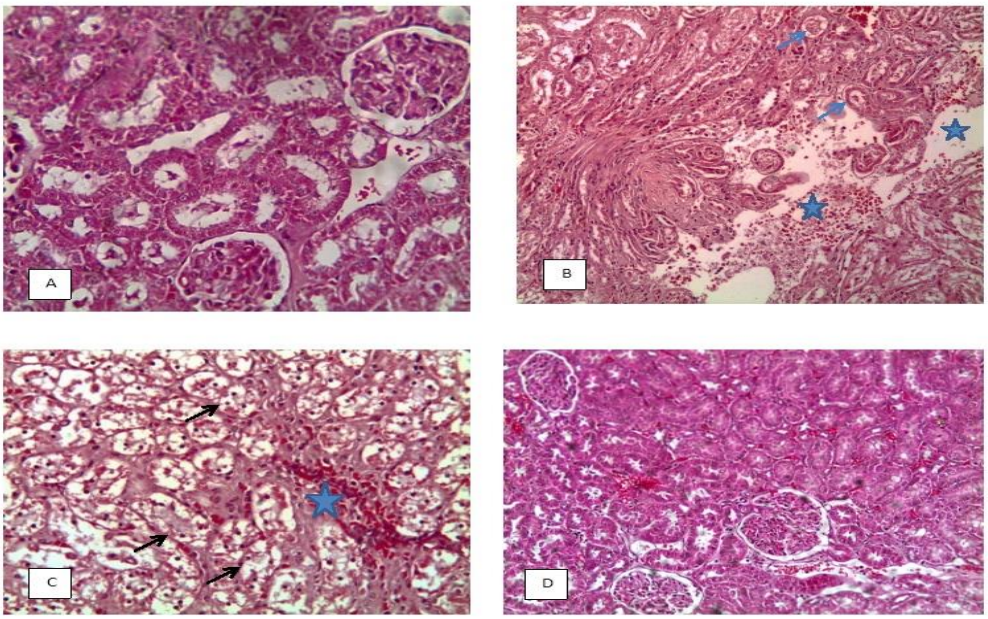

Figure 2:

A: kidney of CG.

B: kidney of TG showing edema and hemorrhage (star), tubular necrosis appearance of cast inside the tubules (arrow), and slight fibrosis.

C: kidney of DAG showing hydropic degeneration, necrosis of tubules (black arrow), edema, and congestion (star).

D: kidney of CAG more or less to normal.

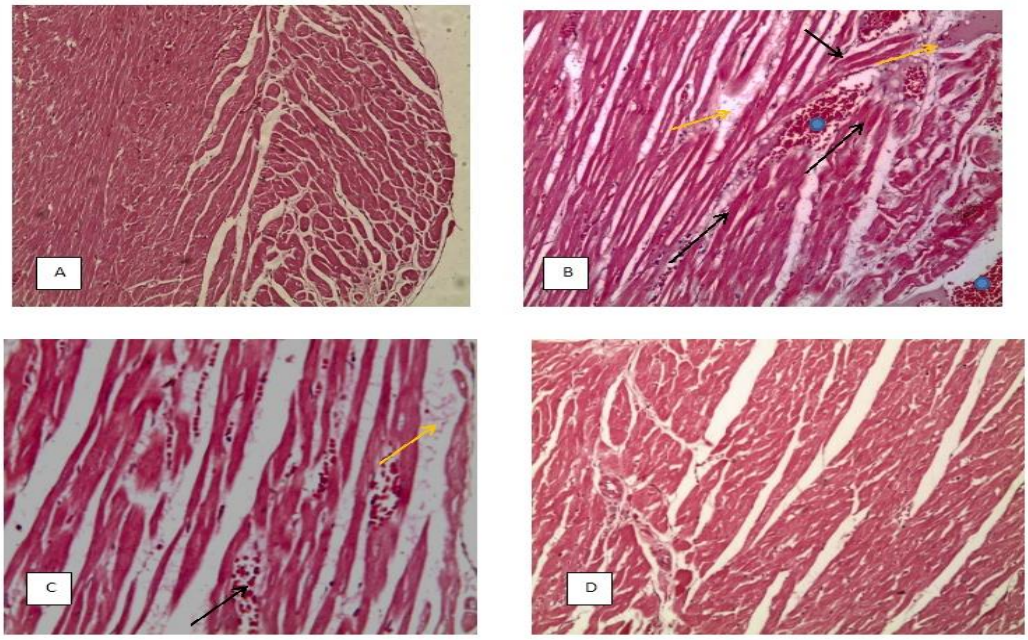

Figure 3:

A: heart of CG.

B: heart of TG showing myocardiolysis, edema (yellow arrow) necrosis of muscle (black arrow), hemorrhage (circle), and infiltration of inflammatory cells.

C: heart of DAG showing myocardiolysis (yellow arrow), hemorrhage (black arrow), and slight swelling.

D: heart of CAG more or less to normal.

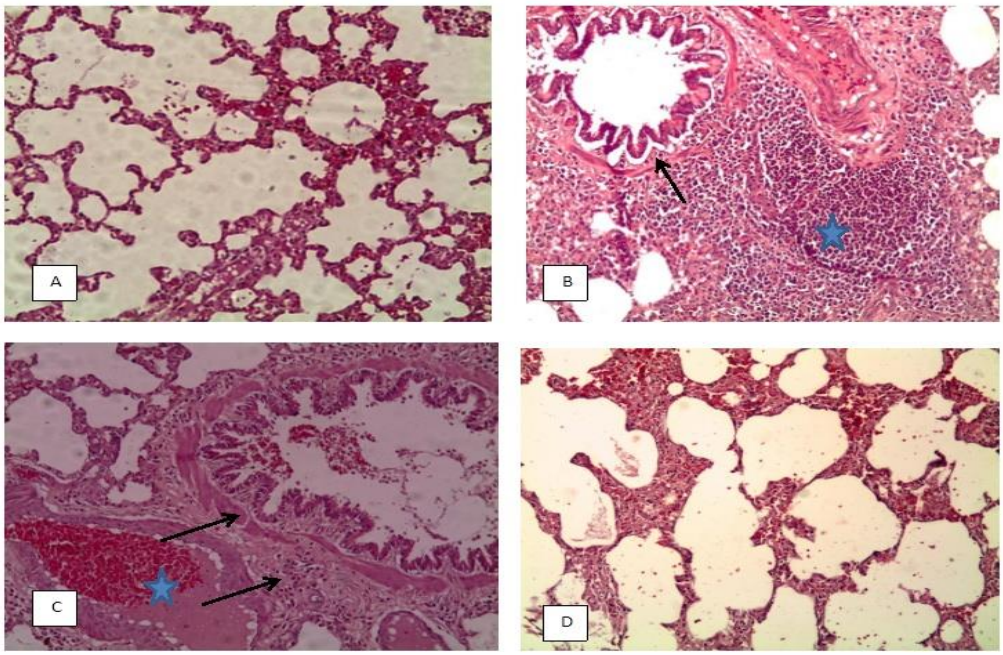

Figure 4:

A: lung of CG showing alveolar emphysema.

B: lung of TG showing bronchitis, peribronchial edema (arrow), and massive infiltration of chronic inflammatory cells (lymphocytic granuloma (star)), and compensatory emphysema.

C: lung of DAG showing catarrhal bronchitis, peribronchial edema (arrow), and slight infiltration of inflammatory cells, congestion, edema (star), and alveolar emphysema.

D: lung of CAG showing alveolar emphysema and hemorrhage.

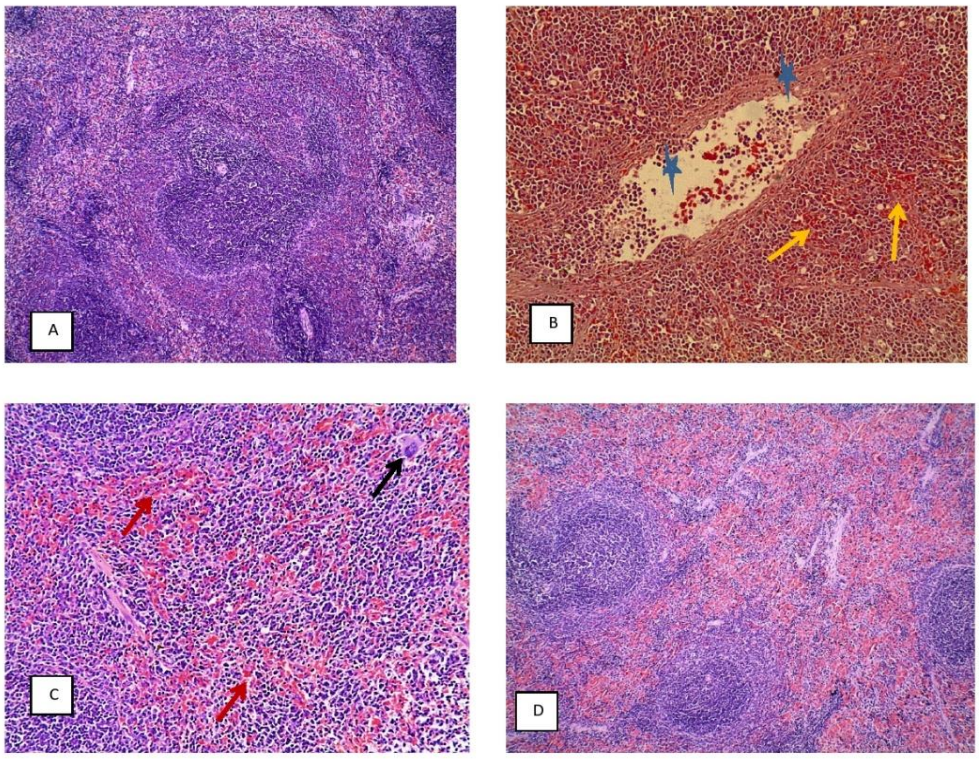

Figure 5:

A: spleen of CG.

B: spleen of TG showing splenic hemorrhage (yellow arrow), vascular and perivascular edema (star shape), and depletion of white pulp.

C: spleen of DAG showing hyperplasia in the splenic lymphoid follicles (black arrow), splenic hemorrhage (red arrow), edema, and depletion of white pulp.

D: spleen of CAG showing more or less to normal.

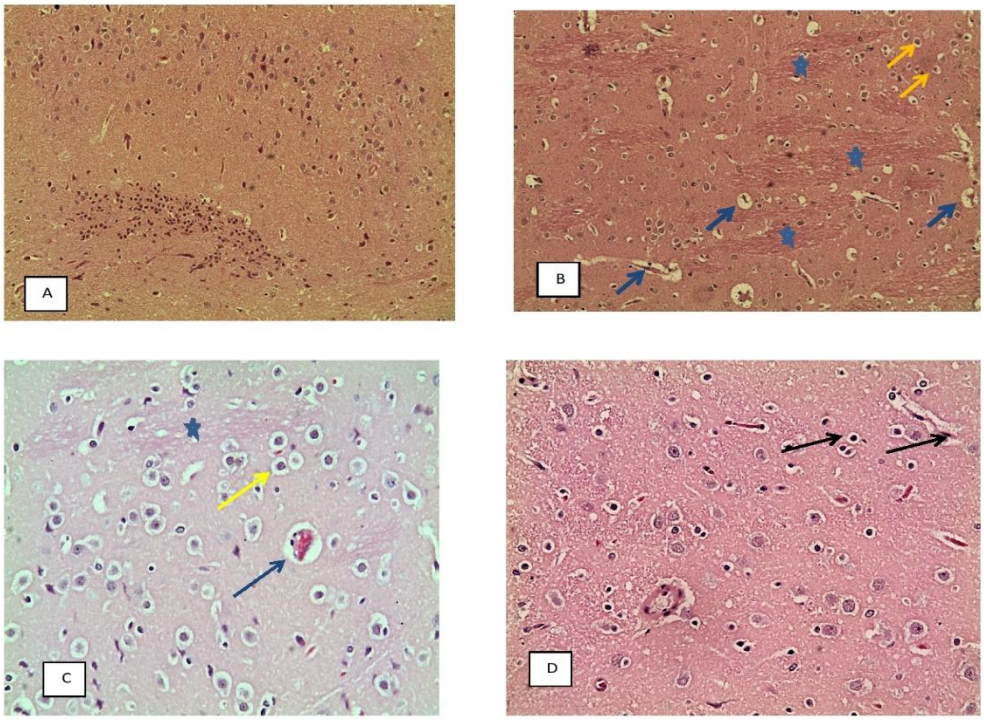

Figure 6:

A: showing brain of CG.

B: brain of TG showing demyelination (star), perivascular edema (blue arrow), chromatolysis of neuron, and neuronal edema (yellow arrow).

C: brain of DAG showing demyelination of brain (star), perivascular edema (blue arrow), chromatolysis of neuron, and neuronal edema (yellow arrow).

D: brain of CAG showing the severity of lesions decreased, neuronal, and perivascular edema appeared (black arrow).

DISCUSSION

Camel trypanosomiasis is a protozoal infection, accompanied with dramatic clinicopathological and pathological alterations. This work as well as previous studies attributed most of these alterations to the oxidative stress related to the disease (represented by the prominent GR depletion in TG) (El-Bahr and El-Deeb, 2016). As, the presence of the mature parasite in the host body as well as the destructed tissue due to its movement, stimulate the pro-inflammatory cytokines liberation from different immune cells. These pro-inflammatory cytokines enhance free radicals' generation to destroy the invading parasite through its cellular component (DNA, lipid, and CHO) oxidation (Baldissera et al., 2016). The anti-oxidant enzymes control the free radicals work and protect the host cells from their oxidative action. In this study, the anti-oxidants (GR) were massively consumed and the free radicals accumulated and reacted with the host cells causing their oxidative destruction (Mohammed et al., 2019). Therefore, TG suffered from an advanced degree of organ damage mainly liver, kidney, skeletal and cardiac muscles, lungs, and brain (Darwish

et al., 2019; Mohammed et al., 2019). This assumption was supported by the high values of hepatic enzymes activities (ALT, AST, and GGT), renal function tests (BUN and Cr), CK (skeletal muscle damage indicator), CK-MB (heart damage indicator), and LDH (lung damage indicator) recorded in TG. In turn, the TG histopathological results, demonstrated a marked degeneration of the examined organs with edema, hemorrhage, fibrosis, and cellular infiltration (Darwish et al., 2019; Mohammed et al., 2019).

The noticed hyperbilirubinemia (T/I/D) in TG here, is another outcome for the oxidative stress. Whereas, the accumulated free radicals react with the RBCs membrane phospholipids causing its lysis. Thus, a large number of RBCs were destructed and I-bilirubin was massively liberated in the host circulation. Besides, the oxidative damage of the hepatocytes inhibits I-bilirubin clearance from the blood and impairs D-bilirubin excretion through the liver (Mbaya et al., 2014; Gopalakrishnan et al., 2019).

The oxidative stress also has a great contribution in the hypoproteinemia (hypoalbuminemia and hypglobulinemia), hypocholesterolemia (T/HDL/LDL/VLDL), and hypoglycemianoted in TG. As the liver is the main factory of albumin, globulin (α, β), and cholesterol. It also regulates the blood glucose levels through glycogenolysis and gluconeogenesis processes. Thus, the above-stated liver oxidative damage hinders protein and cholesterol synthesis and disrupts blood glucose levels (Sivajothi et al., 2015; Eze et al., 2015; Darwish et al., 2019). The oxidative stress also participates in these hypoproteinemia and hypocholesterolemia by another way, as albumin and HDL-cholesterol are potent anti-oxidants and the accumulated free radicals consumed them(Mbaya et al., 2014; Sivajothi et al., 2015; Darwish et al., 2019). The anorexia related to the disease and lack of dietary amino acids, free fatty acids, and glucose are other possible causes for the obtained hypoproteinemia, hypocholesterolemia, hypotriglyceridemia, and hypoglycemia in TG (Mbaya et al., 2014; Sivajothi et al., 2015; Darwish et al., 2019). Furthermore, the parasite depletes the host lipids and glucose due to its opportunistic nature and its inability to create the mandatory lipids and glucose for its survival and reproduction (Mbaya et al., 2014; Sivajothi et al., 2015; Darwish et al., 2019).

Additionally, *T. evansi* disrupts the infected host hemogram resulting in the macrocytic hypochromic anemia recorded in TG in the current work. Previous data referred to the hemolytic origin of this anemia. Whereas the presence of the protozoa in the animal blood induces RBCs mechanical hemolysis and elicits pro-inflammatory cytokines activity (Ahmadi-hamedani et al., 2014; Darwish et al., 2019). The pro-inflammatory cytokines encourage RBCs oxidative damage due to free radicals` liberation and stimulate RBCs splenic sequestration causing a remarkable hemodilution (Mohammed et al., 2019). Besides, the trypanosomal sialidase release in the host circulation causes RBCs membrane hydrolysis and suppresses erythropoiesis. The bone marrow responds to this anemia, via pumping reticulocytes and immature RBCs in the host peripheral circulation. Subsequently, MCV and RDW values increased and the anemia converted from microcytic hypochromic anemia to macrocytic hypochromic anemia (Zewdu et al., 2016; Darwish et al., 2019).

Bone marrow fatigue due to the prior response and trypanosomal enzymes with their toxic effect on leukocytes and thrombocytes are the major reasons for the detected leukopenia and thrombocytopenia in TG (Tribulatti et al., 2005; Adeyeye et al., 2017). Lymphocytic migration during the inflammatory reaction and eosinophilic splenic sequestration (due to pro-inflammatory activity) are extra causes for

the spotted leukopenia in TG here (Adeyeye et al., 2017).

Post-treatment, the parasitological examination of DAG and CAG blood samples were negative and the clinical symptoms of the disease gradually disappeared. As both treatments succeeded in removing the parasite and its toxic enzymes and restoring the animal appetite and counteracting the oxidative stress associated with the disease. Subsequently, the clinicopathological parameters levels of DAG and CAG showed a marked enhancement approaching CG levels.

These results mimicked previous opinions, referred tothe DA direct trypanolytic effect because of its ability to suppress the trypanosomal DNA replication. As, it binds to the trypanosomal kinetoplast DNA (kDNA) in a non-intercalative way and interacts with sites rich in adenine-thymine base pairs (Peregrine, 1994). DA also has anti-inflammatory anti-oxidant action, as it easily modulates the immune response against the protozoa through pro-inflammatory cytokines inhibition. Thus, it reverses the clinicopathological and histopathological alterations associated with the disease (Kuriakose et al., 2012; Kuriakose et al., 2013). On the other hand, chamomile extract showed a potent anti-trypanosomal activity *in-vitro* before. It could dampen the trypanosomal growth within 60 minutes, via disruptingthe parasite cell membrane permeability and targeting its proteins and lipids (Borges et al., 2012; Sobhy et al., 2021). In addition, chamomile essential oilshave anti-inflammatory, anti-oxidant, and anti-microbial properties (Borges et al., 2012; Sobhy et al., 2021).

Interestingly, chamomile was more efficient than DA in controlling the disease consequences and most of its group clinicopathological parameters returned to its physiological ranges and its organs microscopical examination was nearly normal and so close to those of CG. This result was attributed to chamomile extract-rich content of essential oils, mainly bisabolol, which reduced the cytokines storm associated with the disease and related oxidative stress (Borges et al., 2012; Sobhy et al., 2021). On the contrary, DAG had a more pronounced degree of oxidative stress than CAG 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 CAG) (Spinosa et al., 1999; Sobhy et al., 2021). The DAG histopathological results were compatible with these findings, it demonstrated observable degenerative lesions with hemorrhage, edema, and cellular infiltration in the examined organs. Logically, the liver damage explained the hypoalbuminemia (dependent hypoproteinemia and decreased A/G), hypocholesteremia, and hypoglycemia reported in DAG in relation to CG and CAG as mentioned before in this work (Sivajothi et al., 2015; Eze et al., 2015; Darwish et al., 2019). While, the diminished RBCs, WBCs, and PLTs values in DAG pointed to bonne marrow exhaustion and absence of its regenerative response (Adeyeye et al., 2017).

Connecting the CK, CK-MB, LDH, and GR importance in trypanosomiasis diagnosis and prognosis and its treatment monitoring, they are moderate markers and CK-MB is the best among them as a disease marker while, GR is the best in the therapeutic intervention tracking.

CONCLUSION

Chamomileextract is more efficient than DA in *T. evansi* treatment. Among the studied markers, CK-MB is the best for the disease diagnosis and GR is the best for its treatment evaluation. For more accuracy, further field studies on dromedaries are recommended.

CONFLICT OF INTEREST

Authors have no conflict of interest.

AUTHORS CONTRIBUTION

All authors contributed equally

ACKNOWLEDGEMENT

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