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IMPACT OF ROSEMARY (*ROSMARINUS OFFICINALIS*) EXTRACTS ON *TRYPANOSOMA EVANSI* IN EXPERIMENTALLY INFECTED RATS

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Abstract

This study evaluated the activity of rosemary (*Rosmarinus officinalis*) plant extracts on *Trypanosoma evansi* infection compared to diminazene aceturate drug (DA). Eight extracts were prepared at concentrations of 4, 10, & 20mg/ml (four from leaves and four from stem bark) of the rosemary plant collected from Matrouh Governorate. Petroleum ether, ethyl acetate, ethyl alcohol, and distilled water were used in order of increasing polarity. Phytoscreening of those extracts and their toxicity were assessed. *In vitro* & *in vivo* studies evaluated *T. evansi* viability post treatment and the efficiency of different extracts. Also, for potential hematological and biochemical abnormalities that may be associated with the administration of different treatments. The results showed that the phytochemical screening showed significant differences ($p \leq 0.05$) between leaves and stem bark components. Extract of 20mg/ml *in vitro* affected activity of *T. evansi* more than others but, less than diminazene aceturate without acute toxicity. Statistical analysis corroborated anti-trypanosomal activity-specifically correlated to treatment based on solvent type and plant part extracted. *In vivo* results showed a significant reduction in infection-induced alterations in treated groups compared to untreated healthy group. Some extracts did not achieve complete restoration of some selected biochemical indices to a pre-infection state.

Key words: *Trypanosoma evansi*, Rosemary extracts, *In vitro*, *In vivo*, Organic solvents, Rats.

Introduction

Human African trypanosomiasis (HAT) is a neglected tropical disease targeted for elimination as a public health problem by the 2020. Indicators to monitor progress towards the target were based on the number of reported cases, the related areas and populations exposed at various levels of risk, and coverage of surveillance activities (Franco *et al,* 2018).

Trypanosoma evansi is one the most geographically widespread African trypanoso-mes (Abubakar *et al,* 2005; Sobhy *et al,* 2017). It affects a wide range of domestic and wild animals in Africa, Asia, and South America (Ashour *et al,* 2013).

In Egypt, *T. evansi* was reported in man (Haridy *et al*, 2011), camels (Elhaig *et al,* 2013), and farm animals (El-Bahnawasy *et al*, 2014). The infection ranged from the acute disease with high mortality to a chronic one characterized by subcutaneous edema, fever, lethargy, weight loss, abortion, nasal, and/or ocular bleeding (Derakhshan *et al,* 2020). Despite its economic impacts, it was severely neglected awareness, control interventions, and searching for improved control tools (Barghash *et al,* 2018).

The available trypanocidal drugs such as diminazene aceturate®, isometamidium®, and homidium® used in the last two decades, but with development of parasite resistance (Legros *et al,* 2002; Frieri *et al*, 2017). These drugs were old, expensive, less effective, and associated with severe adverse reactions (Capila *et al*, 2019). Thus, medicinal plants with trypanocidal activities continued to generate as indicated need (Atawodi *et al,* 2003). Many studies were done on plant extracts to treat *T. evansi* infection (Nwodo *et al,* 2015; Barghash, 2016; Fathuddin and Inabo, 2017). It attributed the antitrypanosomal activity of some medicinal plants in Africa to their flavonoids, alkaloids, and other phytochemicals constituents (Kabore *et al,* 2010; Mann *et al,* 2010). These metabolites usually were found in various parts of the medicinal plants, roots, leaves, shoots, and/

or stem-bark (Nibret *et al,* 2010).

Lamiaceae family includes many species containing large amounts of phenolic acids, such as rosmarinic acid, with antibacterial, antiviral, antioxidant, and anti-inflammatory properties (Shuaibu *et al,* 2008). One of the species is *Rosmarinus officinalis*, which is an evergreen, wild plant in cold and dry areas and cultivated worldwide as an ornamental and medicinal plant (Atangwho *et al,* 2009). It is also used as food spices, in cosmetic and perfume industries, and as flavoring agents (Kiarostemic, 2010), smooth muscles, and its volatile oil is a growth stimulator (Atangwho *et al,* 2009). Moreover, it has antibacterial, antiviral, antiparasitic, and antitumor activities (Malebo *et al*, 2009; Barghash, 2016; Wang *et al,* 2018; Liu *et al,* 2018; de Oliveira *et al,* 2019).

The present study aimed to screen the phytochemical *in vitro* and *in vivo* efficacy of eight stem and leaf-extracts prepared by different solvents as anti-*Trypanosoma evansi* treatment.

Materials and Methods

Study area: *R. officinalis* was collected from Matrouh Governorate in the northwest of Egypt (31.352778°N, 27.236111°E). It was selected on an ethnopharmacological basis. The study was carried out in the Desert Research Center located in Cairo, Egypt. The plant was identified and confirmed by Taxonomists at the Medicinal and Organic Plants Department. The experimental investigations for *in vitro* and *in vivo* antitrypanosomal activities were performed in the Parasitology experimental Lab of the Animal Health Department.

Plant and solvents used: Mature fresh samples from aerial parts of *R. officinalis* were gathered in March 2018. It is thoroughly washed with water to remove any foreign materials and left to drain off. Then, it air-dried under shade to a constant weight and ground into two separate parts; one for stem bark and the other containing leaves. Petroleum ether, ethyl acetate, ethyl alcohol, and distilled water were used as organic and aqueous solvents, in order of polarity (Harborne, 1998).

Phytochemical analysis: 100mg well-grinding powder from each part of *R. officinalis* was subjected to quantitative phytochemical screening to assess the concentration of alkaloids (Woo *et al,* 1977), flavonoids (Karawya and About, 1982), saponins (Honerlogen and Tretter, 1979), tannins (Van Buren and Robinson, 1969), and total phenols (Snell and Snell, 1953).

Plant extraction: The ground samples weighing 400g of each part were grounded into a powdered form using an electric blender (Kenwood®). They were soaked respectively in petroleum ether, ethyl acetate, ethyl alcohol, and cold distilled water for a week in a continuous extraction apparatus, using the soxhlet apparatus with occasional stirring or agitation on a mechanical shaker (Handa *et al,* 2008). The extracts were filtered through Whatman filter paper No. 1, and each filtrate was evaporated to dryness using a rotary evaporator vacuum at a temperature not exceeding 45˚C. The residue left dried to a constant weight, then weighed and kept at 40^{0C}in separate capped bottles until used. On the experimental day, fresh stock solutions were prepared from residues of each extract at concentrations (20, 10, & 4mg/ml) in the phosphate buffer saline (PBS).

Approval ethics and experimental animals: Healthy female Wistar Rat of 17 weeks old and weighed 200g were purchased from the Faculty of Science, Ain Shams University. They were housed under laboratory conditions, fed a balanced diet, water *ad*-libitum, and allowed to acclimatize for two weeks before the experiment. All rats were handled according to the international guiding principles for Biomedical Research involving Animals and Approved, and conducted with strict adherence to the guideline of the Desert Research Center in Cairo, Egypt.

Parasites: *Trypanosoma evansi* was obtained from the Parasitology Unit, Desert Research Center as passage strain in the Albino rats till parasitemia were 25-32 parasites per

field (Barghash *et al*. (2016).

Anti-*Trypanosoma evansi*: The *in-vitro* activity of eight extracts was performed in triplicates in 96 well microtiter plates (Flow Laboratories Inc.; McLean, Virginia 22101, USA). Forty microliters (40µl) of blood containing about 25-32parasites/field, was mixed with 10µl of each extract solution of 20, 10, & 4mg/ml, respectively.

To ensure the effect of the extract alone, a set of control parasites was suspended in the PBS (pH 7.4) was prepared. Trypaject (DA), a commercial trypanocidal drug was used, each vial contained 5250mg Diminazene aceturate & 6560mg Phenazone (ADWIA Co. S.A.E., Egypt). After 5min. incubation at 37^{o}C, 2µl of the mixture was put on a separate microscope slide, covered with a coverslip, and the parasites motility was observed every 10min. for an hour by Olympus microscope X40. A cessation or drop in *T. evansi* motility in extract-treated blood was compared to control.

Acute toxicity: Up and down procedure of Dixon (1991) was adopted. It started from a minimal dose (LD_{50}) with non-motile significantly reduced *T. evansi*, to maximal dose (LD_{90}) disappearance for each extract using female Swiss Albino rats (3 rats each). Each group was given gradual dose to 1000mg/kg body weight for substance. Also, 3 rats were used as control and received sterile water. Rats were kept under close observation for autonomic, neurological, behavioral, physical changes, and diarrhea for 24hr, with attention during the first 4 hours up to 14 days.

Experimental infection: Ninety female rats were divided into nine groups of ten rats for each in clean cages. They were categorized G1: -ve & G2+ve controls, and G3 was infected and treated with DA (3.5mg/ kg). G4, G5, G6, G7, G8, &G9 were infected and treated with the most suitable concentrations of plant extracts based on the microtiter plate results. Rats were intraperitoneally (IP) injected with the infected blood in PBS (100-µl/ inoculum of 10^5 trypanosomes/ml). The six prepared extracts of 20mg/ml were 4 of leaf and 2 of stem bark. Those extracts were IP injected seven days post-infection at doses started from 500µl/week for at least six successive weeks. Every week, 10ml blood was obtained from each group by heart puncture in two parts on days: 7, 14, 21, 28, 35, 42, 49, 56, & 63 for hematological and biochemical analyses. Sera were stored at 4°C until used (Tab. 1). Parasitaemia was evaluated, and parasites numbers were microscopically counted, by Rapid Matching method (Herbert and Lumsden, 1976).

Table1: Description of experimentally infected and treated groups and the doses were used.

G	Code	Group description	Dose
1	N	-Ve control received PBS only (non- infected non-treated)	0.5 ml/rat/week
2	P	Served as +Ve control (infected non-treated)	100 µl blood/ Rat
3	DA	Served as the drug control (infected and treated with DA)	100 µl /rat /2 times
4	AA	Infected and treated with petroleum ether extract/leaves.	162.5 mg/kg
5	B	Infected and treated with ethyl acetate extract/stem	167.5 mg/kg
6	BB	Infected and treated with ethyl acetate extract/leaves.	162.5 mg/kg
7	C	Infected and treated with ethanol extract/stem	167.7 mg/kg
8	CC	Infected and treated with ethanol extract/leaves.	167.5 mg/kg
9	DD	Infected and treated with aqueous extract/leaves.	162.5 mg/kg

(Note: D and A failed *in vitro* anti-trypanosomal, so canceled from *in vivo*)

The CBCs were automatically done. Biochemical analysis was assessed using commercial kits (Bio-System, Egypt) according to manufacturer's instructions, and an automated chemistry analyzer (BA200, A25 Bio-Systems). Total protein (TP), albumin (Alb), and globulin (Glo), kidney function (Creat), uric acid (UA) were determined. Liver ALT, AST, &ALP were determined. Lipids, like total cholesterol and triglycerides, total bilirubin and glucose were also estimated.

Statistical analysis: Data were collected, tabulated and analyzed using SPSS software program version 20. (M±SEM), the one-way analysis of variance (ANOVA), and LSD Posthoc-tests were applied for the multiple comparisons. P value < 0.05 was considered significant.

Results

Phytochemical analysis: Rosemary extracts varied according to solvent used. It was between 2.053% for ethyl acetate extract/ stem, & 17.14% for petroleum ether extracted leaves. Phytochemical analysis showed the alkaloids, tannins, flavonoids, saponins, and phenols with significant differences ($p \leq 0.05$) between leaves and stem, with high parameters percentages in leaves than stem bark.

Leaves showed highest concentration of saponins, and lowest one was flavonoids. Phenols in stem were high concentration in contrast to alkaloids and flavonoids, but saponins were in low concentrations. Tannin concentration was moderately found in leaf and stem (Tabs. 2 & 3).

Table2: Yields of different solvents in 100gm of each part of plant

Plant part	Weight	Solvent	Yield
Well grinding stem powder	100 g	Extraction of petroleum ether	6.295 g
		Extraction of ethyl acetate	2.053 g
		Extraction of ethanol	3.016 g
		Aqueous extraction	3.195 g
Well grinding leaves powder	100 g	Extraction of petroleum ether	17.142 g
		Extraction of ethyl acetate	4.262 g
		Extraction of ethanol	5.834 g
		Aqueous extraction	4.041 g

Table3: Phytochemical screening of leaves and stem bark of *Rosmarinus officinalis*

Plant part	Alkaloids	Flavonoids as quercetin	Phenols	Saponins	Tannins
Stem powder	0.15%	0.166%	13.5%	0.23%	7.30%
Leaves powder	0.36%	0.675%	22.2%	59.03%	7.75%

In vitro activity: The eight extracts at concentrations 10 & 4mg/ml gave poor effects on trypanosomes with active and motile even after an hour of incubation. By using 20mg/ml, different responses were noticed; about 90% of parasites were immobilized in BB, CC, & DD and cleared from AA, B, & C treatments. They showed slight effects but didn't kill trypanosomes in A &D groups. The standard drug, DA completely immobilized *T. evansi* after 20min incubation and unchanged up to60 min. *T. evansi* in control were active to the experimental end (Fig. 1).

Therapeutic and acute toxicity: Both solvent extracts were safe to infected rats at doses of up to 1000mg/kg, body weight without significant difference ($p \geq 0.05$) were recorded between different experimental groups injected with low and high doses.

Also, no adverse effects or mortality were observed during the two-week follow-up. Besides, no abnormal signs, behavioral changes, body weight changes, or changes in food and water consumption occurred in rats. But, rats become vital and animated, accompanied by more hair growth. No evidence of the acute toxicity at the safety doses.

In vivo activity and parasitemia: Anti-trypanosomal efficacy was evaluated, for the six plant extracts of the suitable concentration tested as compared to healthy and drug-treated controls. The +ve control group exhibited progressive parasitemia resulted in early deaths than others. Drug-treated group relapsed on days 11 & 25 post-infection, and two animals died before the end of the study. More activity happened in B and CC treatments that extended to nine weeks. Significant decreases in RBCs count in all treated rats, except (AA), (DD), were nearly typical compared to healthy group (-ve). Hb in all treatments decreased with significant differences within the normal range in three treatments (AA, B, & DD).

On the contrary the measurements of values WBCs significantly increased in all the treated groups except the two (C & CC). WBCs values also elevated with a significant difference in DD and drug-treated groups compared to healthy control. The other parameters, such as PLT, Lymph%, and HCT, showed that different treatments revolve around the normal range compared to the healthy control group (Tab. 4).

Table 4: Changes in hematological parameters due to treatment

Treat	RBCs (10^6/µl)	HGB (g/dL)	WBCs (10^3/µl)	PLT (10^3/µl)	Lymph (%)	HCT (%)
N	7.99^{a}±.01	13.20^{b}±.06	5.30^{h}±.06	723.00^{b}±.58	87.10^{c}±.06	37.66^{b}± .19
P	4.53^{h}±.01	7.40^{g} ±.06	8.30^{f} ±.06	440.00^{i} ±.58	85.50^{d} ±.06	20.50^{i} ± .09
AA	7.07^{c}±.01	11.89^{c} ±.01	19.20^{a}±.06	502.00^{h}±.58	88.40^{b} ±.06	35.70^{c} ± .09
B	5.33^{f}±.012	11.00^{d} ±.12	10.76^{e} ±.09	551.00^{d}±.58	94.20^{a} ±.06	30.20^{d} ± .09
BB	5.62^{e}±.01	9.30^{f} ±.06	17.20^{c} ±.06	528.00^{f}±.58	63.70^{g} ±.06	24.70^{h} ± .09
C	5.87^{d}±.012	9.40^{f} ±.06	5.80^{g} ±.06	643.00^{c}±.58	71.30^{e} ±.06	26.30^{g} ± .09
CC	5.60^{e} ±.003	6.80^{h} ±.06	1.76^{i} ±.03	1046.0^{a}±.58	53.90^{h} ±.06	26.80^{f} ± .12
DD	7.40^{b}±.01	13.50^{a}±.06	11.00^{d}±.06	506.00^{g} ±.58	85.50^{d}±.06	39.30^{a} ± .06
DA	5.26^{g} ±.012	9.80^{e} ±.06	18.30^{b} ±.06	545.00^{e} ±.58	65.00^{f} ±.06	27.80^{e} ± .06
p	0.000	0.000	0.000	0.000	0.000	0.000

Biochemically, highly significant differences ($p<0.0001$) were among different treatments (Tabs. 5, & 6) compared to drug-treatment (DA) and healthy ones. The inequality levels of glucose, cholesterol, triglyceraldehyde, and hypoproteinemia showed highly significant differences ($p<0.0001$). Aqueous extract of *R. officinalis* (DD) caused reduction with a highly significant difference ($p<0.0001$) in parasite-induced biochemical changes evidenced in decreased LDH, bilirubin, creatinine, cholesterol, levels. Treatment with ethanol and ethyl acetate extracts of stem (C, & B) decreased with highly significant difference ($p<0.0001$) in Tp, Alb, & GLb levels as compared to its pre-treatment levels in all infected groups. Ethanolic leaf extract (CC) was the best treatment that lowered the levels of urea, bilirubin, AST, & ALT liver enzymes with highly significant differences ($p<0.0001$). Petroleum leaf extract (AA) had improved LDH, cholesterol, A/G%, & creatinine levels. But, drug did not improve many parameters, except for WBCs and to less extent, ALP, Tg, TP, Alb, GLb, & A/G.

Table 5: Changes in lipid profile, glucose, and proteins due to treatment

Treat	Lipids		Glucose	Proteins			
	Chol (mg/dL)	TG (mg/dL)	GLU (mg/dL)	TP (g/dL)	Alb (g/dL)	Glb (g/dL)	A/Gratio (%)
N	27.86^{d} ±.34	40.30^{g} ±.12	62.20^{e} ±.06	5.90^{a}±.01	2.06^{c}±.03	2.16^{e}±.03	.953^{b}±.03
P	34.03^{b} ±.09	172.3^{cd}±1.3	56.03^{f}±.03	5.21^{c}±.01	2.40^{b}±.003	2.80^{b}±.00	.856^{cd}±.00
AA	16.73^{g} ±.20	156.50^{e}±2.2	72.20^{d} ±.40	3.59^{h}±.01	1.79^{e}±.003	1.80^{f}±.01	1.00^{a}±.006
B	21.00^{e} ±.12	265.26^{b}±2.2	46.30^{h} ±.12	4.06^{e}±.03	1.89^{d}±.003	2.16^{e}±.03	.876^{c}±.012
BB	13.80^{h} ±.31	170.33^{d}±2.9	50.03^{g} ±.09	3.90^{f}±.01	1.50^{f}±.01	2.40^{c}±.01	.650^{e}±.02
C	32.70^{c} ±.17	50.50^{f} ±.52	113.7^{a}±.09	4.70^{d}±.01	1.89^{d}±.003	2.80^{b}±.01	.673^{c}±.003
CC	38.63^{a} ±.32	320.10^{a}±2.9	75.10^{c} ±.06	3.50^{i}±.01	1.2^{g}±.003	2.30^{d}± .01	.523^{f}±.003
DD	18.70^{f} ±.46	177.10^{c}±.61	50.00^{g} ±.06	3.70^{g}±.01	1.50^{f}±.006	2.20^{e}± .01	.680^{e}±.000
DAS	33.00^{c} ± .17	51.50^{f} ± .58	76.2^{b}±.058	5.70^{b}±.01	2.59^{a}±.01	3.09^{a}±.00	.830^{d}± .003
p	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 6: Changes in liver enzymes and kidney functions due to treatment

Treat	Liver enzymes					Kidney functions	
	AST (pg/ml)	ALT(ng/ml)	ALP(u/l)	Bili (mg/dL)	LDH (u/l)	Urea (mg/dL)	Creat. (mg/dL)
N	69.0^{i} ±.06	25.63^{g} ± .32	166.7^{g} ± .33	.09^{c} ±.01	806.3^{f} ±.33	30.40^{g} ± .06	.50^{h} ±.01
P	154.0^{b}±.18	850.0^{a}±.58	193.0^{c} ±.58	.70^{a} ±.00	1141.2^{d}±.09	56.03^{c} ± .03	1.70^{a} ±.01
AA	98.06^{e}±.03	529.4^{d}±.32	183.7^{e} ±.33	.37^{bc} ±.27	347.7^{i} ±.33	50.30^{e} ± .12	1.60^{b} ±.01
B	108.0^{c}±.29	812.0^{b}±.58	177.7^{f} ±.33	.10^{c} ±.01	634.0^{g} ±.58	60.80^{a} ± .06	1.50^{c} ±.01
BB	93.10^{f} ±.06	223.0^{e}±.58	196.0^{b}±.58	.10^{c} ±.01	1201.0^{b}±.58	60.13^{b}±.033	1.40^{d} ±.01
C	88.13^{g}±.09	24.33^{g} ± .33	187.0^{d}±.58	.20^{c} ±.00	1163.3^{c}±.18	36.40^{f} ± .12	.70^{f} ±.01
CC	77.63^{h}±.32	21.0^{h} ± .58	192.0^{c} ±.58	.10^{c} ±.00	920.0^{e} ±.58	36.50^{f} ± .30	.90^{e} ±.01
DD	104.0^{d}±.00	185.7^{f}±.33	203.0^{a} ±.58	.10^{c} ±.01	506.3^{h} ±.33	52.30^{d} ± .06	.60^{g} ±.01
DAS	173.13^{a}±.1	711.33^{c}±.33	178.7^{f} ±.33	.59^{ab} ±.01	2061.0^{a}±.59	29.06^{h} ± .03	.696^{f} ±.00
p	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Discussion

The use of herbs for treatment of trypanosomiasis still holds strong potential with potent trypanocides (Mbaya and Ibrahim, 2011; Shaba *et al,* 2012; Ibrahim *et al,* 2014). In the European Union, *R. officinalis* was investigated as hepatoprotective, antifungal, insecticide, and other activities such as anti-

bacterial, antimicrobial, and anti-inflammatory properties (Nieto *et al,* 2018; de Oliveira *et al,* 2019) with safety (Hoet *et al,* 2004). Also, it was used as a chemo-preventive agent (Oluwatuyi *et al,* 2004), and has antioxidant activity (Peng *et al,* 2005; Wang *et al,* 2018). Studies evaluated extracts of rosemary as antiplasmodial activity on malaria (Milhau *et al,* 1997), *in vitro* antitrypanosomal against *T. evansi* (Barghash, 2016), and anthelmintic activity on Monogenean infections in *Cyprinus carpio* (Zoral *et al,* 2017). *R. officinalis* extracts proved effective on *Trichomonas vaginalis* and *Candida albicns* (Saeidi *et al,* 2019).
Others found several phenols, flavonoids, and alkaloids with antitrypanosomal activity (Nok, 2002; Barghash, 2016).

In the present study, type of solvent, and the plant part used affected the antitrypanosomal properties. The leaf extracts proved more effective than stem bark extracts but all within the natural concentration (MillogoKone *et al,* 2008). It showed a strong presence of classes of chemical compounds such as flavonoids, alkaloids, tannins, saponins, and phenols compared to stem. These compounds are known to exert pharmacological and antagonistic effects, and some are capable of protecting the active ingredients in herbs from decomposing either chemically or physiologically (Mann *et al,* 2009).

In the present study, LC_{50} of active extracts were higher than the value of the commonly used DA drug, and purification might lead to pure compounds with highly increased activity & no cytotoxicity. The six extracts showed different *in-vitro* antitrypanosomal activity on *T. evansi* due to their ability to lyse and eliminate trypanosomes. Also, to reduce the levels of parasitemia which is suppressed by the most active concentration tested. This agreed with Adeiza *et al*. (2009) although those extracts did not completely clear parasites compared to the DA drug. On contrary, parasites in two stem-extracts (A & D) were large refractory to treatment. That might be due to the extract action being more effective when the parasites have not fulminated in the host (Ibrahim *et al,* 2008).

Studies attributed the *in-vivo* & *in-vivo* activities of *R. officinalis* extracts to the alkaloids, saponins, and phenols which were rich in leaves than in stem amongst others (Nok, 2002; Ogbadoyi *et al,* 2007). Others reported that this may not be unconnected with the presence of phytochemicals as having diverse bioactivities including anti-trypanosomal and antioxidant properties (Adeyemi *et al,* 2009; Akanji *et al,* 2009).

The present study did not involve detailed characterization and isolation of different compounds which may be responsible for the trypanocidal effect, which agreed with Hopp *et al*. (2004). But the differences in the anti-trypanosomal activity of some extracts were observed in the current study compared to previous studies may be resulted from the part of the plant used for extraction, as previously explained by Muregi *et al*. (2003). Also, the quantitative differences *in-vitro* activity may be due to variation in chemical composition arising from different solvents (Atawodi *et al*, 2003). Besides, the crude extracts have a very complex combination. At this stage, it is not possible to identify the compounds that could be responsible for the observed activities because of the complex composition of our present extracts.

The present *in vivo* study showed that the metabolic disposition of bioactive constituents of rosemary differed between *in vivo* & *in vitro* conditions in some extracts (Antia *et al*, 2009). Mechanism of these organic and aqueous extracts exert their trypanocidal activity is unknown since the active ingredient(s) are not isolated. DA-drug likes pentamidine and other diamines could interfere with polyamine synthesis and suramin target glycolysis in glycosomes (Atawodi *et al,* 2003). Consistent suppression of parasitemia was observed with prolonged survival time of treated rats in the *in vivo* study by 200 mg per kg bodyweight of *R. officinalis* extracts. This effect was lower than DA drug-treat.

The dose associated with its ability to improve the observed decreases in their hematological parameters was due to its ability to decrease parasite load. The early deaths observed in the infected control group may result from massive parasitemia that induced hemolytic anemia as found in *T. evansi* & *T. b. brucei* (Atawodi *et al,* 2003; Barghash, 2016, 2020). The severity of trypanosome infection was correlated with parasitemia (Adeyemi *et al*, 2009). The RBCs destruction caused anemia in zoonotic *T. b. brucei* and *T. evansi* (Anosa, 1988; Igbokwe *et al,* 1994; Barghash, 2020). In the present study, comparison between haemogram parameters in healthy and treated rats showed cure of anemic induced by *T. evansi* infection by normal level of RBC count, HGB, WBCs, PLT, Lymph%, & HCT in DD than others.

In the present study, the increased in liver enzyme levels were reported in *T. brucei* & *T. evansi* (Ibrahim *et al,* 2008), and none was able to improve liver enzymes (Barghash, 2016). Some *T. evansi* treatments in the rats caused significantly ($P< 0.001$) increased serum levels of AST, ALP, ALT, & LDH (Umar *et al*, 2007), but significantly ($P < 0.001$) decreased in others (Al-Otaibi *et al*, 2019). Also, inconsistent changes in serum total bilirubin concentration in infected animal model progressed for stem-bark extracts due to acute hemolysis resulting from the proliferating parasites (Orhue *et al,* 2005).

In the present study, decrease in serum total protein was due to progressive infection caused by a drop in serum albumin level due to liver failure. Also, it could be due to decreased release of other intracellular proteins consequent upon parasite-induced cell membrane disruption (Orhue *et al*, 2005). Also, the decreases in serum albumin levels may be due to malabsorption (Guyton and Hall, 2000). This agreed the fact that trypanosome infections have a strong link to plasma expansion, proteinuria, & hepatocellular damage (Anosa, 1988; Tijani *et al,* 2009). Besides, the decreased glucose serum concentration may be due to abnormalities in liver functions, metabolism and anorexic state of affected animals (Sazmand *et al,* 2016). Disparity of decrease and increase in serum lipid caused hyperglyceridemia and hypocholesterolemia was reported (Bala *et al,* 2012). Biu *et al*. (2013) found that trypanosomal continued utilization of lipid molecules from the bloodstream as a source of energy. This inconsistency in biological and biomarkers caused by non-isolation, fractionation, and characterization of extracts pure compounds was reported (Sasidharan *et al,* 2011).

Conclusion

The rosemary extracts showed potential antitrypanocidal effects with very low toxicity to a safety degree. The *in vitro* and *in vivo* activities depended mainly on the solvents used and part of the plant extracted. Some rosemary crude extracts possessed *in vivo* activity to different degrees against *T. evansi* but didn't prevent the liver damage, with high significant differences in all hematological and biochemical parameter. However, the leaf of rosemary might be a promising treatment of *T. evansi*.

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Explanation of figure

Fig.1. *In vitro* antitrypanosomal activity of rosemary extracts at 20mg/ml concentration with time. 4 stem extracts (A, B, C&D) & 4 leaf extracts (AA, BB, CC& DD) showed different activities against *T. evansi*. AA (petroleum ether extract/leaf) and B (ethyl acetate extract/ stem), followed by C (ethanol extract/stem) most impact whereas A (petroleum ether/stem) and D (aqueous extract/ stem) least values.