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RESEARCH ARTICLE

EVALUATION OF IN VITRO AND IN VIVO ACTIVITIES OF SOME MEDICINAL PLANTS EXTRACTS AGAINST TRYPANOSOMIASIS.

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Abstract

This study aimed to assess the antitrypanosomal activity of leaf ethanolic extracts against *Trypanosoma evansi* experimental infection. Seven medicinal plants: *Camellia sinensis*, *Thymus vulgaris*, *Mentha longifolia*, *Azadirachta indica*, *Olea europaea*, *Rosmarinus officinalis* and *Salvia officinalis*, were selected based on information from traditional healers on their curative effect against parasites. They were investigated for phytochemical screening and antitrypanosomal properties. Results revealed the presence of saponins, tannins, flavonoids, alkaloids, and phenols. The *in vitro* antitrypanosomal activity in microtiter plates showed that all of them had pronounced trypanocidal effects and inhibited the growth of *T. evansi* that was observed either disappeared, dissected or non-motile within 10-60 min. of incubation except for *O. europaea* and *R. officinalis* which could not clear the parasites completely. Therefore, only five plants were evaluated for their *in vitro* extracts administration and *in vivo* against *T. evansi* experimental infection. Haematological analysis of the infected and treated rats revealed a marked decline in hemoglobin, hematocrit, total red blood corpuscles, and anaemia persisted until the end of the experimental period. Whereas, the biochemical assay showed increased serum levels of urea, creatinine, aspartate aminotransferase and alanine aminotransferase, total bilirubin, and decreased albumin, globulin, total protein, cholesterol, triglycerol and glucose in some treated groups. We concluded that *S. officinalis* followed by *C. sinensis* possess high antitrypanosomal activity compared to diminazene aceturate, and could be useful in the management of trypanosomiasis. Further study needed to claim and illuminate the phytochemical compounds responsible for this antitrypanosomal activity.

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Introduction:-

Animal trypanosomiasis constitutes one of the major veterinary problems that spread beyond its original distribution in sub-Saharan Africa to North Africa (Atarhouchet *et al.*, 2003; Barghash *et al.*, 2014; Omer *et al.*, 2013). One of the important pathogenic trypanosomes in animals is *Trypanosoma evansi*; the etiological agent of Surra that is may rapidly fatal or take a chronic course continues for several years in domestic mammals (Vanhollebeke *et al.*, 2006). Trypanocidal drugs constitute the principal method of control, as the development of vaccines against

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trypanosomiasis is still in progress. The current drugs used as veterinary trypanocides include isometamidium chloride, diminazene aceturate, quinapyramine, and cymelarsan. The existing treatments of trypanosomiasis are challenged with problems comprising drug resistance, toxicity and expensive/limited drugs (Atawodi *et al.*, 2002; Legroset *al.*, 2002). Thus, there is an urgent need for new antitrypanosomal drugs that are more effective and safer.

Previous studies reported that some medicinal plants, especially in Africa, have trypanocidal activity due to their flavonoids, alkaloids, and other phytochemicals constituents (Kaboreet *al.*, 2010; Mann *et al.*, 2010). The use of herbal preparations for the treatment of Surra disease still holds a strong potential in that some ethnomedicinal plants have been demonstrated to contain potent trypanocides (Atawodi *et al.*, 2005; Ibrahim *et al.*, 2014; Mbaya and Ibrahim, 2011; Shaba *et al.*, 2012). Several secondary plant metabolites including alkaloids, tannins and anthraquinones have been indicated with trypanocidal activity (Hoppet *al.*, 2004). This presents an urgent need to develop efficacious chemotherapeutic agents from locally available ethnomedicinal plants for use trypanocides. This is the first work had been conducted in Egypt to evaluate the trypanocidal activity of some medicinal plant extracts in compared to diminazene aceturate (DA) and to assess the *in vitro* and *in vivo* antitrypanosomal activity for these extracts using rats as a biological model. It was also carried out to assess potential Haematological and biochemical abnormalities that may be associated with the administration of crude leaf ethanol extracts against trypanosomiasis.

Material and Methods:-

Plant samples collection:-

The leaves of seven medicinal plants screened: *Camellia sinensis*, *Thymus vulgaris*, *Mentha longifolia*, *Azadirachta indica*, *Olea europaea*, *Rosmarinus officinalis* and *Salvia officinalis*. They have collected from different locations on the northern west coastal zone of Egypt except for green tea originated from China was purchased from Libya market at Mersa Matrouh, Egypt (Fig.1). Some of these plants were traditionally used in the treatment of parasitic and microbial diseases. They were identified and confirmed by Taxonomists at Medicinal and Organic Plants Department, Desert Research Center, Egypt, and then investigated for *in vitro* and *in vivo* antitrypanosomal activities.



Fig. 1 Showing the medicinal plants (encoded from A to G) were selected for *in vitro* and *in vivo* antitrypanosomal activity.

Preparation of Extracts:-

The leaves of the collected plants were air-dried to a constant weight and grounded into powdered form using an electric blender (Kenwood®). They were extracted by the Soxhlet. The ethanolic extract for each was made by soaking 20g of each powdered plant material in a solution of 200 ml of 95% ethanol, following prior extraction with petroleum ether to de-fat the sample, then filtered using Whatman no. 1 Filter paper. The obtained filtrates were evaporated gently to dryness on a water bath set at 45°C. The residues were weighed and used for administration by dissolving in Tween 80 (0.3% v/v), then stored in airtight containers in a refrigerator until required.

Phytochemical Screening of Extracts:-

The presence of phenols, saponins, tannins, flavonoids and alkaloids in the crude extract were determined according to Parekh *et al.* (2005).

Trypanosome propagation:-

T. evansi was isolated from a naturally infected camel at El-Bassatein abattoir (Cairo, Egypt), then identified and maintained in the laboratory by the continuous passage in white Swiss mice of mixed sexes (20–25 g) to give parasitaemia of approximately 10^5 parasites/ml. They were infected IP with 0.01 ml of blood containing about 1×10^3 trypanosome cells. The blood film prepared from tail blood at x40 magnification was used as an estimation of parasitaemia. The trypanosome count was determined using the "rapid matching" method of Herbert and Lumsden (1976).

In vitro test for trypanocidal activity of leaf plant extracts:-

Assessment of the in vitro antitrypanosomal activity was performed in triplicates in 96 well microtiter plates (FLOW LABORATORIES INC., MCLEAN, VIRGINIA 22101, USA) according to Atawodi *et al.*, 2009 with some modifications. Approximately, 40 μ l of blood containing about 50-100 parasites per field was mixed with 10 μ l of each leaf extract solution of different concentrations (120, 60, 30, 20, 10, 5, 2, 0.5, 0.01 and 0.001 mg/ml). The control was the parasite suspended in 0.3% Tween 80 only, while the commercial trypanocidal diminazene aceturate (DA) used as a reference. After 5 min incubation at 37°C, about 5 μ l of test mixtures were placed on slides and covered with coverslips. The parasites observed every 10 min. for a total duration of sixty minutes. Motility and change in morphology of *T. evansi* occurred under the microscope (x40) were recorded, and complete elimination of motility or reduction in parasite count was taken as a significant activity of the extracts.

Antitrypanosomal activity of the extracts in rats:-**Animal model:-**

Females Swiss Albino rats (Albino Wister) weighing between 200 to 220 gm were purchased from National Research Center, Egypt, and kept in well ventilated plastic cages. They were exposed to 12 hours light and dark cycles fed with pellets and fresh vegetables and watered ad libitum throughout the experimental period. The experiment was conducted in compliance with international accepted principles for laboratory animal use. The rats were allowed a 7-day period of acclimatization before they were divided into groups of ten rats per cage. The weights of the rats were measured before and after treatments till the end of the experiment.

In vitro test for extract administration:-

Out of seven plants used, only five were selected based on their primarily in vitro antitrypanosomal activity against *T. evansi*. Eleven different groups with four Swiss Albino rats each was coded and distributed into 11 cages. Ten groups were classified into two groups for each plant at the two most suitable concentrations based on microtiter plates results: one for the minimal dose (LD₅₀) that non-motile and significantly reduced *T. evansi*, and the second for the maximal dose (LD₉₀) disappeared *T. evansi*. They were IP administered different extracts at doses of 500 μ l per week for two successive weeks. The remaining group consisted of the negative control (without leaf extract).

In vivo assay and experimental design:-

Eight different groups (n=10) was distributed into 8 cages. Of which, five groups (encoded from A to E) were classified into one group for each selected plant extract at its suitable in vitro concentration and administration. The sixth group (T) composed of infected but not treated control. These groups were infected with 0.1 mL of blood from infected mice containing 3×10^5 trypanosomes, were intraperitoneally administered extract at doses of 500 μ l/200gm body weight for two successive weeks, starting a day 5 post infection after the parasites were detected in the blood stream. The seventh infected group (DA) was given intraperitoneally about of 50 μ l (after serial dilution of 0.5 ml/10 Kg body weight) of diminazene aceturate® to give reference values. The other group (N-N) maintained an uninfected untreated control.

Therapeutic monitoring of extract:-

On the seven-day post infection and after two days of extracts and drug administrations, the parasitaemia level of rats in the controls and extracts groups were checked three times a week by wet blood film prepared from tail blood at x40 magnification. Rats were observed daily for clinical signs that included weight loss, edema of the face, ocular discharges, encrustation of the lips, lethargy and mortality rate. Prepatent period, longevity and animal mortality were also observed. The number of parasites seen per field under the microscope was counted and treatment efficacy was determined by the number of rats that did not show clinical signs post-treatment against *T. evansi* infection. Blood was collected once a week until the end of the experiment for Haematological and biochemical parameters. The whole blood and serum were kept at -20°C for analysis.

Haematological and biochemical analyses:-

Every 7 days, 5 mL blood samples were obtained from each group by heart puncture in two parts with and without anticoagulant, for a period of 42 days. All sera samples were stored at 4°C until used. For the hematology analysis, the microhematocrit technique (HCT), red blood cells count (RBCs), total white blood cells count (WBCs), platelets and hemoglobin (Hb, Drabkin's method) of the whole blood were done manually. Mean corpuscular haemoglobin concentration (MCHC) were calculated from the results obtained from RBC, HB, and HCT according to Tornquist (2010). Biochemical analysis was done with fully automated analyzed using commercial kits (SPECTRUM, EGYPT) according to manufacturer's instructions. Liver enzyme (alanine) aminotransferase (ALT), Aspartate aminotransferase (AST), Total protein, Albumin, Globulin, Cholesterol, Glucose level (Liquizyme), Triglyceride (Liquizyme) were estimated as per the instructions of the manufacturer. Serum Creatinine was measured by the Jaffe' method while Urea were estimated by Liquizyme method.

Statistical analysis:-

Data obtained were expressed as mean±standard error of the mean. It was analyzed using SPSS version 20.0 (IBM SPSS Statics 20, USA). Data were subjected to one-way analysis of variance (ANOVA) and the post-hoc test was applied for multiple comparisons. Values of P <0.05 and P <0.001 were regarded as statistically significant and highly significant, respectively.

Results:-**Phytochemical screening of the ethanol extracts:-**

The results of the phytochemical screening in Table 1 revealed the presence of tannins, saponins, alkaloids, phenols, and flavonoids with different values ranged from high to low. *C. sinensis* (A) gave high yield, the great value of tannins, saponins, and phenols, and the least value of alkaloids and flavonoids. Results also showed that *S. officinalis* (B) has the least values of tannins and phenols, while *Th. vulgaris* (C) gave the least value of flavonoids and yield.

In vitro antitrypanosomal activity:-

Results of the *in vitro* antitrypanosomal activity of extracts screened in microtiter plates revealed that all of them showed high activity against *T. evansi* parasites that observed either disappeared, dissected or non-motile within 10-45 min. of incubation, except for F and G which lasted more time to reduce the parasites at higher concentrations and could not clear the parasites completely. The minimum inhibitory concentration of the extracts from A to E against *T. evansi* parasites were between 0.001 mg/ml and 0.01 mg/ml. The standard drug (DA) immobilizes the parasite after 15 minutes, while the control still showed very active motile parasites in 0.3% Tween 80 at the end of 60 minutes. The motility, reduction, and disappearance of *T. evansi* were directly proportional to the concentrations of the plant extracts. On the other hand, *in vitro* extracts administration revealed that LD₅₀ were 78.5, 26.5, 59.0, 30.0, 40.5 mg/ kg body weight, while LD₉₀ were 300, 99.5, 102, 52.0, 150 mg/ kg body weight for plants from A to E, respectively. It also demonstrated that the two selected doses for each plant were safe for rats (Table 1). The combination of these results made LD₉₀ of each plant extract the best option for processing *in vivo* study.

In vivo antitrypanosomal activity:-

All *T. evansi* experimentally infected groups gave parasitaemia of approximately 10⁵ parasites/ml at day 5 post infection. After extract administrations, groups from A to E showed parasite clearance from 3th-day to 11th-day post-treatment, except for group B that took two days more to kill *T. evansi* that increased in numbers throughout this time before clearance. At the 22th-day post-infection, *T. evansi* were detected and progressed to high numbers for 4 days followed by a reduction in numbers ranged from 1 to 4 trypanosomes/ field before complete disappearance on 30th-day post-infection. The parasites returned as non-motile on day 33 then disappeared followed by a relapse, *T. evansi* were detected in all groups 2 days before the end of the experiment, however, a number of rats in groups D and E died on the 27th day as a result of reappearance and progress of parasites. The *in vivo* experiment showed rats infected and not treated (group T), died after 17th-day post-infection. In contrast, IP injections of DA at 3.5 mg/kg body weight (group AD), completely cleared the parasite from the circulation day 1 after the first dose and returned on day 10. The second dose was able to clear the parasites from circulation with prolonging survival period of up to 25 days. Group N-N (not infected not treated) was healthy till the end of the experiment. The extracts of the five plants from A to E exhibited antitrypanosomal effects at 60, 20, 20, 10 and 30 mg/ 200 gm body weight, respectively (Table 1).

Table 1:- Phytochemical screening of the seven selected medicinal plants and their yields, names, and *in vitro* antitrypanosomal activities

Leaf plant extract (Code)	Family	Common name	Yield	K	N	O	S	P	In vitro Antitrypanosomal activity	
									LD ₅₀	LD ₉₀
<i>Camellia sinensis</i> (A)	Theaceae	Green tea	2.15	0.14	10.9	0.15	19.73	27	78.5	300
<i>Salvia officinalis</i> (B)	Lamiaceae	Sage	1.43	0.15	2.4	0.41	7.78	5	26.5	99.5
<i>Thymus vulgaris</i> (C)	Lamiaceae	Thyme	0.4	0.54	7.2	0.13	7.31	8	59.0	102
<i>Azadirachta indica</i> (D)	Meliaceae	Neem	1.33	0.20	2.9	0.24	7.59	7	30.0	52.0
<i>Mentha longifolia</i> (E)	Lamiaceae	Wild mint	2.44	0.30	12.45	0.24	14.81	23	40.81	150
<i>Olea europaea</i> (F)	Oleaceae	Olive	1.02	0.50	7.5	0.18	20.67	21	Excluded from <i>in vivo</i> study due to weak results (reduced parasites but not significant) at higher concentrations	
<i>Rosmarinus officinalis</i> (G)	Lamiaceae	Rosemary	0.22	0.33	3.6	0.16	14.27	9		

Analysis of total alkaloids (K), tannins (N), flavonoids (O), saponins (S) and phenols (P) was mg/ 100gm, while minimal and maximum lethal doses (LD₅₀ and LD₉₀) were mg/ kg body weight, as they have antitrypanosomal activity against *T. evansi* based on the results of *in vitro* micro titer plate and extract administration. Both doses were safe in rats, but only LD₉₀ was used *in vivo* antitrypanosomal study.

Clinical Observation:-

Animals of group T (infected but not treated) exhibited typical clinical signs included oedema in the face, ocular discharges, and the poor condition after experimental infection with *T. evansi* started from day 5 post-infection. It was significantly higher till death than all treated groups (DA, A-E) and N-N control group. In addition, the weight loss was observed in all infected and treated groups with no significant difference. Anatomically, no significant difference was observed between extracted treated groups and DA drug treated group. However, different tissues from those groups showed pathological changes in compared to N-N (healthy group). Gross post-mortem examination revealed enlargement of the spleen (splenomegaly) in all groups except group N-N, hemorrhages in lungs (in groups D, E), and very slight enlargement of the liver in the terminal stages of disease just before the end of the experiment. The heart and liver appeared with slight changes in color in all groups, but few abscess were observed only in D group by the end of the fourth week after treatment.

Haematological and biochemical analyses:-

The RBCs counts were found reduced in infected and treated groups as compared to the healthy group (N-N), while Hb was found significantly lower ($P < 0.05$) in all infected groups except for group B that within the normal value. The HCH values were within the normal range in all infected and treated groups except groups A, C, E that significantly decreased ($P < 0.05$). Results of the calculated MCHC revealed a decrease in infected treated groups and the N-N group without significant differences. On the other hand, WBCs values were within normal range in all control groups (N-N, T, DA) and C treated group, whereas it was significantly higher in groups A, B, D and E than other groups. The platelet's counts were within normal in all groups except for groups DA, A, B, and D that found decreased non-significantly. Results are shown in Table 2.

Table 2:- Mean \pm Standard Error of the hematological values of experimentally infected and treated groups in compared to control groups in rats.

Groups	RBCs	HB	HCT	MCHC	WBCs	PLTs
N-N	7.72 \pm .27 ^b	15.37 \pm .525 ^b	47.36 \pm 1.55 ^b	32.44 \pm .19 ^a	8.52 \pm 1.05 ^{abc}	743.33 \pm 74.71 ^a
T	5.85 \pm .36 ^{ab}	11.31 \pm .73 ^a	35.38 \pm 2.65 ^a	32.04 \pm .27 ^a	6.68 \pm .78 ^{a,b}	615.40 \pm 246.70 ^a
DA	6.59 \pm .67 ^{ab}	13.34 \pm 1.24 ^{ab}	41.02 \pm 3.73 ^{ab}	32.50 \pm .07 ^a	6.41 \pm .89 ^a	565.10 \pm 99.55 ^a
A	6.64 \pm .78 ^{ab}	12.16 \pm .79 ^a	37.46 \pm 2.37 ^a	32.44 \pm .06 ^a	9.20 \pm 1.16 ^{abc}	544.00 \pm 83.23 ^a
B	6.88 \pm .75 ^b	13.86 \pm 1.28 ^{ab}	42.63 \pm 3.83 ^{ab}	32.48 \pm .08 ^a	11.12 \pm 2.27 ^{abc}	565.94 \pm 80.92 ^a
C	6.06 \pm .30 ^{ab}	12.36 \pm .59 ^a	38.11 \pm 1.80 ^a	32.43 \pm .034 ^a	6.96 \pm .84 ^{ab}	630.60 \pm 110.13 ^a
D	6.43 \pm .03 ^{ab}	12.90 \pm .15 ^{ab}	39.70 \pm .45 ^{a,b}	32.49 \pm .01 ^a	12.91 \pm 3.94 ^c	587.00 \pm 230.03 ^a
E	4.75 \pm .66 ^a	11.19 \pm 1.14 ^a	34.56 \pm 3.43 ^a	32.34 \pm .10 ^a	11.81 \pm 2.43 ^{bc}	678.50 \pm 90.05 ^a
F-test	2.21	2.54	2.46	1.14	2.11	.30
P value	.061	.035	.039	.365	.072	.950

Significant ($P < 0.05$); highly significant ($p < 0.001$)

Results of biochemical analysis are represented in Table 3 showed high significant differences ($P < 0.001$) observed in the levels of glucose, cholesterol, triglyceride, urea, creatinine, ALT, AST, total bilirubin, total protein, albumin, and globulin ranged from high to low in infected and treated groups compared to the control groups. Also, it proved and evidenced that group B has the ideal biochemical values compared with other groups and consequently, the ethanolic plant extract of *S. officinalis* is a promising antitrypanosomal medicinal plant against *T. evansi*. It followed by group A that gave values within the range in all parameters except ALT, AST that high increased significantly ($P < 0.001$), and albumin which slightly decreased. Despite the availability of DA as *in vitro* and *in vivo* antitrypanosomal drug, it gave some abnormal high values in urea and ALT, and low levels of albumin. Results also revealed that groups C, D could be used as trypanocidal but not *in vivo* antitrypanosomal studies.

Table 3:- Mean \pm Standard Error of the biochemical values of experimentally infected and treated groups in compared to control groups in rats.

Parameter	Control groups			Infected and treated groups					F-test
	N-N	T	DA	A	B	C	D	E	
Glucose	122.67 \pm 5.8 ^{6c}	66.2 \pm 8.73 ^a	86.6 \pm 4.85 ^b	114.67 \pm 5.67 ^c	117.8 \pm 4.70 ^c	154.4 \pm 3.50 ^d	67.0 \pm 2.74 ^a	65.3 \pm 5.78 ^a	31.2
Cholesterol	41.17 \pm 1.11 ^c	21.80 \pm 2.71 ^a	30.60 \pm 3.37 ^b	53.00 \pm 1.83 ^d	64.80 \pm .74 ^e	33.0 \pm 3.96 ^b	22.75 \pm 1.11 ^a	103.0 \pm 2.89 ^f	93.0
Triglyceride	39.83 \pm 5.89 ^b	15.50 \pm 2.00 ^a	21.60 \pm 2.48 ^a	88.00 \pm 5.19 ^c	132.0 \pm 1.10 ^e	23.4 \pm 1.72 ^a	22.75 \pm 3.92 ^a	162.0 \pm 8.51 ^a	150.9
Urea	20.67 \pm .80 ^a	47.20 \pm 10.30 ^c	26.60 \pm 3.14 ^{ab}	17.67 \pm 2.11 ^a	13.00 \pm .45 ^a	41.6 \pm 2.21 ^c	36.5 \pm 6.85 ^{bc}	37.7 \pm 4.33 ^{bc}	7.4
Creatinine.	.36 \pm .01 ^a	1.18 \pm .29 ^d	.57 \pm .05 ^{ab}	.46 \pm .04 ^a	.32 \pm .01 ^a	.96 \pm .09 ^{cd}	.64 \pm .07 ^{abc}	.93 \pm .15 ^{bcd}	7.31
ALT	30.33 \pm 1.98 ^a	112.40 \pm 35.91 ^b	55.80 \pm 8.78 ^a	57.33 \pm 5.31 ^a	30.60 \pm .87 ^a	111.8 \pm 2.38 ^b	70.5 \pm 6.06 ^{ab}	71.0 \pm 6.4 ^{ab}	5.52
AST	92.00 \pm 10.2 ^{ab}	474.0 \pm 133.7 ^d	174.6 \pm 25.89 ^{abc}	192.8 \pm 16.4 ^{abc}	72.60 \pm 1.44 ^a	304.0 \pm 8.28 ^c	249.8 \pm 34.3 ^{bc}	217.3 \pm 21.6 ^{abc}	6.4
Total Bilirubin	.1967 \pm .003 ^a	.2840 \pm .034 ^b	.23 \pm .02 ^{ab}	.2017 \pm .01 ^a	.18 \pm .004 ^a	.400 \pm .03 ^c	.38 \pm .03 ^c	.280 \pm .05 ^b	14.2
Total Protein	5.82 \pm .10 ^{de}	4.82 \pm .23 ^b	5.24 \pm .25 ^{bc}	5.50 \pm .13 ^{cd}	5.98 \pm .04 ^e	4.02 \pm .12 ^a	4.78 \pm .076 ^b	4.87 \pm .07 ^b	18.2
Albumin	3.72 \pm .09 ^a	2.66 \pm .12 ^a	3.22 \pm .22 ^a	3.58 \pm .14 ^b	3.79 \pm .01 ^b	2.70 \pm 06 ^b	2.20 \pm 15 ^b	3.37 \pm .12 ^c	21.4
Globulin	2.10 \pm .05 ^a	2.16 \pm .12 ^a	2.02 \pm .05 ^b	1.92 \pm .04 ^b	2.19 \pm .04 ^b	1.32 \pm 07 ^b	2.58 \pm 09 ^b	1.50 \pm 06 ^c	31.1

There is a high significant ($p < 0.001$) between and within groups at the same horizontal line.

Discussion:-

Trypanosomiasis is one of the major parasitic diseases for which control is still far from reality (Toya, 2010). Due to the wide geographic range of Surra that spread beyond its original distribution in Africa, its control has attracted international attention for achieving a decrease in mortality and morbidity using biomedical treatment (Aksoy, 2003; Enwezor and Sackey, 2005). In the present study, the Egyptian selected plants were found to have a strong trypanocidal activity confirmed by *in vitro* and *in vivo* antitrypanosomal studies. These plants have been identified belong to *Lamiaceae* and *Meliaceae* families. According to their phytochemical analyses, it revealed the presence of bioactive compounds such as tannins, saponins, alkaloids, phenols, and flavonoids that may responsible for their antitrypanosomal activity as reported by Janakiraman *et al.*, 2012.

Many studies have shown that plants with *in vitro* antitrypanosomal activity may have no effect *in vivo*. To obtain an additional evidence for the antitrypanosomal activity in the current study, we carried out *in vitro* study for pre-selection of plants followed by the *in vivo* study. The *in vitro* trypanocidal activity of the studied extracts varied from immobilization, reduction and disappeared trypanosomes in corresponding ELISA plate wells. This may be due to these extracts inhibited *T. evansi* exist in higher and lower concentrations levels. Therefore, it is taken as a relatively reliable indicator of the viability of *T. evansi* when compared to the controls and considered promising in the antitrypanosomal screening of crude plant extracts in compliance with Atawodi and Ogunbusola, (2009). The higher value observed for the DA despite its low concentration could be explained by the pure nature of the chemical compound. While plant extracts still crude and impure may lead to antagonistic action of the component compounds in the extracts. Despite this, all crude extracts from A to E exhibited *in vitro* antitrypanosomal activity against *T. evansi*. They were considered most active when inhibited 90% of parasites before 25 min. of incubation compared with F and G extracts. Moreover, the extracts administration gave direct evidence for their safety and gave the choice in selecting the suitable concentration for the start *in vivo* study, in particular; no symptoms of toxicity have occurred for all groups.

In the present study, the reappearance of *T. evansi* on the eleven-day post treatment might be a dose-dependent reduction in the parasitaemia of rats with IP of plant extracts causing the elevation of anaemia and weight loss directly proportional to parasitaemia levels (Coustouet *et al.*, 2012). These constant features of trypanosome infections provide information on the severity of the infection. The significant decrease in HCH, HB, and RBCs values of *T. evansi* infected rats in groups T, A, C, E was in accordance with earlier reports (Faremi and Ekanem, 2011; Oduola *et al.*, 2010) in trypanosome-infected animals. This may be due to the release of hemolytic factors into the animals blood by dead trypanosomes caused a destruction of erythrocytes and HB, and hence reduction in HCH (Olukunle *et al.*, 2010). The normal value in HCH after treatment in groups DA, B, and D may be due to the ability of these extracts to eliminate parasites from the blood (Eghianruwa, 2012; Umar *et al.*, 2007). Anaemia was more severe in control and treated groups except for group B that suffered from slight anaemia as compared to non-infected non-treated rats. It may attribute to intravascular hemolysis as supported by Herrera *et al.* (2002). The weight loss observed in the infected groups is in agreement with the reports of Abubakar *et al.* (2005) and Coustou *et al.* (2012), in which infection with *T. evansi* was associated with weight loss in mice and rats. The significantly increasing of the total leukocytes in all five treated groups (except group C), may be due to the multiplicity of the antigens resulting from more stimulation of immune system (Faremi and Ekanem, 2011) or due to the breakdown of RBCs by phagocytic cells to clean up the toxic remnants of ruptured red blood cells (Olukunle *et al.*, 2010).

Although hypoglycemia is one of the major markers of trypanosomal infection result in increased catabolism of lipids and cholesterol for energy needs (Vigueira *et al.*, 2012) in groups D and E, no change in glucose levels associated with trypanosomiasis in treated groups. The present study also revealed a decrease in serum lipid caused hyperglyceridemia and hypocholesterolemia in *T. evansi* experimentally infected rats in some groups. These findings are in agreement with other previous studies (Adam *et al.*, 2009; Bala *et al.*, 2012; Biuet *et al.*, 2013) who explained the lowering of the serum triglycerol and cholesterol as a result of trypanosomal continuous utilization of the lipid molecules from the blood stream as an important source of energy. On the other hand, the hyperglyceridemia and hypercholesterolemia in group E of wild mint may be due to defective plasma triglyceride degradation that probably caused free fatty acid unavailable for importation into hepatocytes despite serum triglyceride elevation (Igbokwe *et al.*, 2009). The elevated values of enzyme assays ALT and AST activity in the control and treated animals except for groups N-N and B could be attributed to necrosis and inflammation in the liver, heart and kidneys of the host or probably results from host destruction of trypanosomes as previously reported (Abubakar *et al.*, 2005; Takeet and Fagbemi, 2009). In addition, there was a significant difference in the serum albumin, globulin, total bilirubin and total protein of infected and treated animals compared with N-N group could be due to the difference in the

antitrypanosomal activities of selected plant extracts in contrary to Hilaliet *al.*, 2006 who reported increase in the total protein during trypanosomiasis. The elevated urea and creatinine levels in the *T. evansi*-infected rats could be due to the destruction of kidney cells resulting in the inability of the kidneys to excrete creatinine during the course of the disease as previously reported (Abd El-Baky and Salem, 2011; Boniface *et al.*, 2011). The increase in bilirubin may be due to hemolytic anaemia or the inability of the liver to conjugate bilirubin as explained by Adeyemiet *al.* (2012).

Although *M. longifolia* and *A. Indica* demonstrates a wide range of antibiotic activity against various bacteria, yeasts, and insects and were promising plants have *in vitro* antitrypanosomal activity in the current study. It has been shown that these plants are not *in vivo* safe. It gave fluctuating abnormal biochemical ranged between high and low values. It also raised the liver activity even at the safest doses administered in the rat in agreement with Mikailiet *al.*, 2013. Therefore, these natural insecticides have raised levels of liver enzymes that may cause liver injury, toxicity, and reflect the increase in the severity of liver damage with time as in agreement with Bello *et al.*, 2014. In addition, group E of *M. longifolia* gave cytotoxic high values in all parameters except in albumin and globulin that increased significantly. Administration of the standard drug (DA) and plant extracts of B followed by A prevented the disease-induced increases in serum. This may be due to that liver and kidneys could not have been affected at the time of experiment in A and B groups. Some of these findings in the present study are in accordance with the previous reports (Bello *et al.*, 2014; Gutierrez *et al.*, 2013; Muhammad *et al.*, 2013).

Conclusion:-

In conclusion, this study has established the *in vitro* and the *in vivo* antitrypanosomal activity of some medicinal plants, and its potential as a possible drug candidate in the management of trypanosomiasis may be attributed to its high antioxidant activity in spite of the exact mechanism of action is unknown. The data collected indicate that *Salvia officinalis* can serve as promising antitrypanosomal agent. Further study needed to purify and determine the phytochemical compounds responsible for this antitrypanosomal activity.

Ethical approval:-

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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