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POSSIBLE ANTISCHISTOSOMAL AND ANTINENOCEPTIVE ACTIVITY OF DIFFERENT EXTRACTS OF *Glycerrhiza Glabra*

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ABSTRACT:

Glycyrrhiza glabra is one of the most known medicinal plants; it showed wide range of biological activities. In this study both methanol and chloroform extract were evaluated for antischistosomal and antinenoceptive activities. Parasitological studies of plant extracts in *S.mansoni* infected mice showed that treatment with chloroform extract gave the best result when compared with methanol extract, it reduced the total number of portomesenteric worms to be 1.67 Vs 11.5 to methanol extract. The total number of worm was reduced by 62.33 Vs 22.08 %. The total number of coupled worms were reduced by chloroform extract was 73.92 Vs 28.29. Treatment with chloroform extract was highly reduced the total number of *S.mansoni* eggs by about 62.41 % and the total number of dead ova was increased to be 19.32 % and the total number of immature ova was reduced to be 31.08. Chloroform extract also showed more analgesic effect than methanol extract. A phytochemical analysis and chromatographic isolation were carried on both extract.

Keywords: *Glycyrrhizaglabra*, antischistosomal, antinenoceptive, *S. mansoni*, isolation.

INTRODUCTION

Schistosomaisis is still one of the most prevalent epidemic diseases in Egypt, it affects between 200 and 300 million people in 79 countries. Egyptians have a long history of symptoms caused by schistosomiasis, notably haematuria[1]. In endemic areas, repeated chemotherapy has resulted in the emergence of drug resistance strains of schistosomes. The development of such resistance has drawn the attention of many researchers to alternative drugs of plant origin. Natural products in general and medicinal plants in particular, are believed to be an important source of new chemical substances with potential

therapeutic efficacy. Taking into account the most important analgesic prototypes (e.g. salicylic acid and morphine) were originally derived from the plant sources, the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic and antiinflammatory drugs.

Licorice is derived from the roots and rhizomes of *Glycyrrhizaglabra*plant (family Fabaceae), the plant has a history of consumption for the past 6000 years. It is sweet, moist, soothing herb. In traditional medicine it used as antiulcer [2,3], antitumor [4], antibacterial[5] and antimicrobial agent [6], it also used as flavoring sweetening agent, demulcent and protective action for hepatotoxicity[7].

This study investigates antischistosomal and antinenoceptive activity of methanol and chloroform extracts of *Glycyrrhiza glabra* phytochemical analysis and isolation of their chemical constituents.

MATERIALS AND METHODS

2.1. Equipment

^{1}H-NMR (δ [ppm], *J* [Hz]) and ^{13}C-NMR spectra were recorded in CD_3OD, operating at 300 MHz for proton and 75 MHz for carbon 13 spectrometer. Chemical shifts (δ) are reported in parts per million, using TMS as internal standard. Mass spectra were recorded on a finnigan TSQ 700 GC/MS equipped with a finnigan electrospray source (ESI-MS). Paper chromatography sheet [Whattman 1], using 15 % Acetic acid as solvent system, the chromatograms were visualized under UV light (at 254 and 366 nm). Column chromatography was performed using a glass column 120 x 7 cm and using polyamide and silica gel as a stationary phase.

2.2. Extraction

The dry powder of licorice plant (2 Kg) were purchased from the local market, samples were ground and 1Kg were separately soaked in 70 % methanol and chloroform for two weeks (4 L X 3) each, and then evaporated under reduced pressure using rotatory evaporator to give 50 gm. of crude methanol extract and 26 gm. of chloroform extract.Both extract wassubjected to chromatographic fractionation.

30 gm. of the crude methanol extract was subjected to polyamide column chromatography eluted with water, water: Methanol with gradient ratios, then pure methanol. Fractions (500 ml each) were collected and the similar fractions grouped together according to paper (PC) and/or TLC chromatogram. Five groups [I-V] were collected. The first group [I] contains sugar and traces of undetected compounds. Compounds 1& 2 were isolated from group II (7.5 gm.) with elution system 30 % Methanol. The group III (1.5 gm.), (50% methanol) gives compound 3. Compound 4 was isolated from group IV (2.3 gm.) with elution system 80 % methanol. Compound 5 was isolated from group V (1.9 gm.) with pure methanol.

12 gm. of the crude chloroform extract was subjected to silica gel column chromatography eluted with chloroform, chloroform-methanol with different ratios then finally with methanol to give three fraction groups [A-C]. Six compounds were isolated from chloroform extract. Compounds 6-7 were isolated from fraction A (1.5 gm.) with elution system $CHCl_3$: Me OH 80:20. Compound 8-10 obtained from fraction B (0.9 gm.) With elution system $CHCl_3$: Me OH 60:40. Compound 11was isolated from fraction C with pure methanol. All the isolated compounds were known.

2.3. Evaluation of acute toxicity of different extract of Glycerrhiza

Chloroform and methanol extracts of Glycerrhiza each was orally administered to a group of mice male and female in a dose of 0.1- 4.0 g/kg body weight was given once. The behavior parameters observed after administration were convulsion, hyperactivity, sedation, grooming, and increased or decreased respiration during a period of seven days. Food and water were provided ad libitum.

2.4. **Determination of LD50**

Aqueous solution of each plant extract in a dose of 25, 50, 100, 200, 400, 1000, and 2000, 3000, 4000 mg/kg body weight respectively was given once. Mortality and morbidity of animals were recorded 24 hours later. Results were analyzed statistically and LD50 values were calculated according to Litchfield and Wilcoxon., 1949[8].

2.5.**Evaluation of the antischistosomal activity**

2.5.1.Animals and Infection

Male C57Bl/6 mice (18-20g), bred and maintained at the Schistosome Biological Supply Center (SBSC), Theodor Bilharz Research Institute (TBRI) Giza, Egypt were used in this study. Animals were infected using body immersion technique according to the method of Liang et al. (1987)[9] with 80 ± 10 cercariae/mouse.

2.5.2.Drugs and dosages

1-**Praziquantel**, (Distocide, E.p.I.C.O. Pharmaceuticals, Cairo, Egypt), was given orally as an aqueous suspension in 2% Cremophor EL (Sigma Chem. Co., St. Louis, MO, USA) 7 weeks post infection in full dose (500 x 2 mg/ kg**) [10].**

2- **Chloroform and methanol extracts of licorice** each was prepared using 2% Cremophor EL and orally administered to animal in 200 mg/kg for 2 weeks after infection with S. mansonicercariae.

2.5.3. **Animal groups**

After animal infection, animals were divided into 4 groups. First group received the vehicles of the given drugs (infected control group), second group received praziquantel. Third group received the chloroform extract of licorice. The fourth group received methanol extract of licorice.

2.5.4**Experimental design**

All animal groups were killed by decapitation, blood was collected and serum was separated by centrifugation at 3000 rpm for 10 min and used for determination of some biochemical parameters. Perfusion of animals was done according to Duvall and Dewitt (1967) using a MasterFlex pump (Cole-Parmer Instrument Company). Worms recovered from hepatic and portomesenteric veins were collected, counted, and classified according to sex. Parts of both the livers and intestines were removed and kept frozen for subsequent study of tissue egg load[11].

2.6.**Determination of some biochemical parameters**

- **Determination of serum alanine- amino transferase (ALT) and Aspartate-amino transferase (AST)**

Serum ALT and AST was determined using Boehringer reagent kit, Mannheim, Germany according to the method of Reitman and Frankel (1978)**[12].**

- **Determination of serum total protein and albumin, globulin and A/G ratio**

Serum total protein was determined according to the method of Gornall et al.(1949)[13] and albumin according to the method of Bartholomew and Delany (1964)[14]. Globulin was calculated by subtraction of albumin from total protein. Serum concentration of albumin divided by concentration of globulin called A/G ratio.

2.7.**Evaluation of antinenoceptive activity**

2.7.1.*Animals*

Male Swiss albino CD-1 mice (6- 8 weeks old) and were obtained from Schistosome Biology Supply Center (SBSC), Theodor Bilharz Research Institute, Giza, Egypt, and were housed under suitable laboratory conditions through the period of investigation. Animals were fed standard pellet chow (El-Nasr chemical Co., Cairo, Egypt) and allowed free access to tap water**.**

2.7.2.***Drugs and dosages***

- Morphine sulfate was given intra peritoneal in a dose of 10mg/kg (El-Nasr Pharmaceutical Co., Cairo, Egypt)**.**

- Aspirin was given orally in a dose of 100mg/kg (Alexandria Pharmaceutical Co. Cairo, Egypt).
- **Chloroform and methanol extracts of licorice** each was prepared using 2% Cremophor EL and orally administered to animal in a dose of 200 mg/kg.

2.7.3.***Acetic acid-induced writhing in mice***

Acetic acid (0.6% v/v, 10ml/kg) was injected into the peritoneal cavities of mice, which were placed in a large glass cylinder, and the intensity of nociceptive behavior was quantified by counting the total number of writhes occurring between 0 and 20 min after stimulus injection, as described earlier[15]. Oral treatments with vehicle, indomethacin, Chloroform and methanol extracts of licorice were given 1h prior to acetic acid injection (n = 6 per group). Morphine sulphate was intraperitoneally administered (i.p.) 30 min before the test. The writhing response consists of a contraction of the abdominal muscle together with a stretching of the hind limbs. The antinociceptive activity was expressed as writhing scores over a period of 20 min.

2.7.4.**Hot plate test**

The hot plate test was used to measure the response latencies according to the method described previously by Eddy and Leimback (1953)[16] with minor modifications. In this experiment, the hot plate (UgoBasile, Model-DS37) was maintained at 55 ± 0.2 ^{o}C. The reaction time was noted by observing either the licking of the hind paws or the jumping movements before and after drug administration. The cut-off time was 20 s and morphine sulfate 10mg/kg (El-Nasr Pharmaceutical Co.) was administered intraperitoneally and was used as reference drug[17].

2.7.5.**Tail immersion test**

The lower two-thirds of the tail were immersed in a beaker containing water kept at 50±0.5 ◦C [18]. The time in seconds until the tail was withdrawn from the water was defined as the reaction time. The reaction time was then measured 0, 60, and 120min after the oral administration of vehicle, Chloroform and methanol extracts of licoriceand morphine (*n* = 6 per group), with the reaction time of zero minutes being the start of the test. The mice were exposed to hot water for no longer than 20 s to avoid tissue injury.

2.8.**Statistical analysis**

The data obtained were analyzed using the Graph Pad software program Version 4.0 and expressed as a mean ± S.E. Statistically significant differences between groups were calculated by the application of an analysis of variance (ANOVA) followed by the Newman–Keuls test. *P-Values* less than 0.05 ($p < 0.05$) were used as the significance level.

RESULTS

3.1. Identification of the isolated compounds

licochalcone B [1]:

Positive ESI-MS : 287

[M+H]+, 255. ^{1}H NMR (300 MHz, DMSO): 3.5 (3H, s, OMe), 6.7 (1H,d, J=8Hz, H-6), 6.8(2H, d, J=8Hz, H-3'& H-5'), 7.22 (1H, d, 8.Hz, H-5), 7 .56 (1H, d, J=15Hz, H-) 7.9(1H, d, j=15Hz, H-), 8.1 (1H, d, J=8 Hz, H-5).

isolicoflavanol [2]:

Positive ESI-MS : m/z 355

[M+H]+. ^{1}H NMR (300 MHz, DMSO): 1.45, 1.6 (6 H, s, 2 Me), 6.1 (1H,t , J=7Hz, H-2''), 6.22 (1H, d, J=2Hz, H-6), 6.55(1H, d, J=2Hz, H-8), 7.01(1H, d, J=8Hz, H-5'), 7.91 (1H, d, J=2, 8 Hz, H-6'), 7.99 (1H, d, J= 2 Hz, H=2').

Licorice saponin L 3 [3]:

Positive ESI-MS : m/z 985.80

[M]+ , 706.87, 530.0, 528.4. ^{1}H NMR (300 MHz, DMSO) 0.89, 0.96, 1.02, 1.16, 1.33, 1.43 (3H, S), 1.46 (3H, d, Rhmn.), 4.95 (1H, d, J=7 Hz, H-1'), 5.5 (1H, d, J=7.0Hz, H-1''), 5.4 (1H, Br s, H-1'''). ^{13}C NMR .

Isoliquirtin [4]:

Positive ESI-MS : m/z 418

[M]+ , ^{1}H NMR (300 MHz, DMSO): 6.3(1H, d, J=2Hz, H-3'), 6.5(1H, dd, J= 8. 2 Hz, H-5'), 6.8

(2H, d, J=8 Hz, H-3, 5), 7.5 (1H, d, J=15 Hz, H-), 7.7(2H, d, J=8 Hz,H-6), 7.8 (1H, d, J=15 Hz, H-), 8.1 (1H, d, J=8Hz, H-6').

Licoagrochalcone D [5]:
Positive ESI-MS : m/z 354[M]+, ^{1}H NMR (300 MHz, DMSO): 1.15 (3H, s, 5"-CH3), 1.2(3H, s, 4"-CH3), 3.33 (1H, dd, J=16, 9.5Hz, 1"-Ha), 3.30 (1H, dd, J=16, 9.5Hz, 1Hb), 3.4 (3H,s,OCH3), 4.2 (1H, dd, 10.0, 8.5 Hz, 2"-H), 4.5(1H, dd, J=10, 8.5Hz, 2"-H), 6.5 (1H, d, J 8.5, 5-H), 7.0 (2H, d, j=8.5 HZ, 3' and 5'-H) 7.6 (1H, d, J+16.0 Hz, α-H), 7.7)1H, d, J=8Hz, 6-H), 8.1, (1H, d, J=16.0, β-H), 8.6 (2H, d J= 8 Hz, 2', 6'-H). ^{13}C NMR: 189.2 (C=O), 122.3 (C-α), 142 (C-β), 120.1 (C-1, 160 (C-2), 119.2 (C-3), 166.6 (C-4), 105.0 (C-5), 131.1(C-6), 131.5 (C-1'), 131.6 (C-2'), 114.8 (C-3'), 162.6 (C-4'), 115.0 (C-5'), 132.0 (C-6'), 30.0 (C-1"), 93.4 (C-2"), 73.3 (C-3"), 24.6 (C-4"), 25.9 (C-5"), 60.2 (OCH3).

Lup-20(29)-en-3β-ol (lupeol) [6]:
Positive ESI-MS : m/z 426
[M]+, ^{1}H NMR (300 MHz, DMSO): 1.6 (H-30), 1.1 (H-26), 0.9(H-23), 0.89(d, H-27), 0.8(H-25), 0.77(H-24). ^{13}C NMR: 39.4 (C-1), 26.9 (C-2), 80.6 (C-3), 39.4 (C-4), 55.9 (C-5), 19.0 (C-6), 35.8 (C-7), 40.9 (C-8), 50.7(C-9), 35.9(C-10), 20.9 (C-11), 26.1(C-12), 38.0 (C-13), 40.7 (C-14), 25.5 (C-15), 34.6(C-16), 44.2(C-17), 46.7(C-18), 46.5 (C-19), 90.6 (C-20), 30.3 (C-21), 40.0 (C-22), 29.0 (C-23), 16.3 (C-24), 16.5 (C-25), 16.1 (C-26), 15.0 (C-27), 18.0 (C-28), 111.7 (C-29), 19.9(C-30).

P-Coumaric acid [7]:
Positive ESI-MS : m/z 166
[M]+, ^{1}H NMR (300 MHz, DMSO): 12.1 (1H, s), 9.1 (1H, s), 7.51 (1H, d, J=16.0 Hz), 7.2 (1H, d, J=8.0Hz), 6.8 (1H, d, J=8Hz), 6.9(1H, d, J=2.5 Hz), 6. 70 (1H, dd, J= 8, 2 Hz), 6.2 (1H, d, J=16.0Hz). ^{13}C NMR: 17.7, 165.7, 146.9, 138.5, 131.8, 122.4, 120.2, 116.8, 116.3.

Protocatechuic acid [8]:
Positive ESI-MS : m/z 153
[M]+, ^{1}H NMR (300 MHz, DMSO): 7.33 (1H, d, J=2.0 Hz, H-2), 7.23 (1H, dd, J=8.0, 2.0 Hz, H-6), 6.78 (1H, d, j=8.0, H-5). ^{13}C NMR: 166.9 (C=O), 121.7 (C-1), 115.8 (C-2), 145.6 (C-3), 155 (C-4), 116.0 (C-5), 122.5 (C-6).

Kaempferol [9]:
Positive ESI-MS : m/z 285
[M]+, ^{1}H NMR (300 MHz, DMSO): 7.7(2H, d, J=8.0 Hz, H-2', H-6'), 6.4 (2H, d, J= 8.0 Hz, H-3', H-5'), 6.2 (1H, d, J=2.0 Hz, H-8), 6.05, d, J=2.0 Hz, H-6).^{13}C NMR: 155.0 (C-2), 130.9 (C-3), 175.5 (C-4), 160.0 (C-5), 94.3 (C-6), 163.7 (C-7), 91.0 (C-8), 154.2 (C-9), 103.5 (C-10), 120.0 (C-1'), 132.4 (C-2'), 116.1 (C-3'), 165.3 (C-4'), 116.3 (C-5'), 133.3 (C-6').

Quercetin [10]:
Positive ESI-MS : m/z 303
[M]+, ^{1}H NMR (300 MHz, DMSO): 6.1 (1H,d, j=2.0Hz, H-6), 6.3 (1H, d, 2.0Hz, H-8), 6.9 (1H, d, J=8.0Hz, H-5'), 7.5 (!H, dd, J=8, 2 Hz, H-6'), 7.8 (1H, d, J=2.0 Hz, H-2'). ^{13}C NMR: 156.5 (C-2), 132.2 (C-3), 179.5 (C-4), 161.3 (C-5), 98.6 (C-6), 165.7 (C-7), 93.9 (C-8), 157.0 (C-9), 105.5 (C-10), 122.2 (C-1'), 116.4 (C-2'), 146.1 (C-3'), 145.4 (C-4'), 116.7 (C-5'), 123.6 (C-6').

Cinnamic acid [11]: Positive ESI-MS : m/z 149
[M]+, ^{1}H NMR (300 MHz, DMSO): 7.4 (1H, d, J=16, H-α), 6.5 (1H, d, J=16Hz, H-β), &.4 (2H, m, H-2, H-6), &.2 3H, m, H-3, H-4, H-5). ^{13}C NMR: 175.9 (C=O),147.1 (C-α), 117.9 (C-β), 134.7 (C-1), 125.6 (C-2), 126.6 (C-3), 135.3 (C-4), 126.9 (C-5), 128.3 (C-6).

Table 1 Effect of treatment with different extracts of Glycerrhizaglabrata on worm burden and sex in S. mansoni infected mice at 7 weekspost infectionand sacrificed 9 weeks post infection.

Animal groups	Total no. of worms in hepatic	Total no. of worms in portomesenteric	Total no. of males	Total no. of females	Total no. of couples	Total no. of worms	% Worm reduction
Infected Control	4.**67**±0.41	21.00±1.31	13.00±0.97	12.67±1.05	7.67±0.56	25.67±1.65	----
Praziquantel (1000mg/kg)	0.67±0.33	0.17±0.17*	0.67±0.21*	0.17±0.17*	0.0±0.0*	0.83±0.30*	96.77
Methanol extract of Licorice (400mg/kg/2weeks)	8.5±0.22*	11.5±0.70*	9.50±0.22*	10.50±0.22	5.50 ± 0.20	20.00±0.44*	22.08
Chloroform extract of Licorice (400mg/kg/2weeks)	8.00±0.36*	1.67±0.21*	5.33±0.21*	4.33±0.56*	2.00±0.0*	9.67±0.42*	62.33

*Significant difference from infected control at p<0.05.
Values given are means ± SE

Table: 2. Effect of treatment with different extracts of Glycerrhizaglabrata on oogram pattern egg/gm tissue in liver and intestine in S. mansoni infected mice at 7 weeks post infection and sacrificed 9 weeks post infection.

Animal Groups	(Oogram Pattern) % Egg developmental stages			Number of ova per gram tissue (X 10^3)			
	Total immature ova	Mature ova	Dead ova	Liver	Intestine	Total Number of Ova	% Ova Reduction
Infected Control	40.**50** ± 4.28	46.97 ± 2.93	12.53 ± 3.05	11.78 ± 1.02	26.72±2.36	38.5 ± 2.55	--
Praziquantel (1000mg/kg)	0 ± 0*	0 ± 0*	100 ± 0*	2.62 ± 0.71*	1.17 ± 0.30*	3.79 ± 0.92*	90.18
Methanol extract of Licorice (400mg/kg/2weeks)	38.17±5.28	45.45±3.53*	16.38±5.77*	8.43±1.21	19.20±1.62	27.63 ± 2.78	28.23
Chloroform extract of Licorice (400mg/kg/2weeks)	31.08±1.53	49.6±3.81	19.32±3.84*	4.77±0.92	9.70 ± 0.92	14.47 ± 0.98	62.41

*Significant difference from infected control at p<0.05.
Values given are means ± SE.

Table: 3.Effect of treatment with different extracts of Glycerrhizaglabrata on some biochemical parameters compared with praziquantel in S. mansoni infected mice at 7 weeks post infection and sacrificed 9 weeks post infection.

Animal Groups	ALT (U/L)	AST (U/L)	Total Protein (g/L)	Albumin (g/L)	Globulin (g/L)	A/G ratio
Normal Control	59.0±5.4	76.0±2.7	6.3±0.3	3.47±0.11	2.8±0.2	1.2±0.1
Infected Control	123.3 ± 6.5# ((108.9))	167.0±6.1# ((119.7))	6.1±0.2	2.75±0.15#	3.4±0.2#	0.82±0.1
Praziquantel (1000mg/kg)	72.0 ±3.7* (41.6)	95.5 ±3.6#* (42.81)	6.3±0.2	3.35±0.12	2.9±0.3	1.2±0.1
Methanol extract of Licorice (400mg/kg/2weeks)	84.1±2.2#* (31.79)	121.0±4.6#* (27.54)	6.2±0.3	3.20±0.12	3.0±0.4	1.1±0.1
Chloroform extract of Licorice (400mg/kg/2weeks)	79.1±1.1#* (35.8)	118.5±4.5#* (29.04)	6.3±0.4	3.28±0.15	3.0±0.4	1.2±0.1

*Significant difference from infected control group at P< 0.05.
Significant difference from normal control group at P< 0.05.
Values given are means ± SE, ( ) % change from infected control group., (( )) % change from normal control group.

Table: 4. Antinociceptive activity of some of different extracts of Glycerrhizaglabratacomparing with reference drug (aspirin) using acetic acid-induced writhing test.

Animal Groups	Dose of drug	Writhing number (Count/20min) Mean± S.E	% Inhibition	% Analgesia
Control (Acetic acid 0.7%/saline)	0.01ml/g	66.67 ± 1.91	-------	--------
Methanol extract of Licorice	400 mg/ kg	24.67±5.43*	62.99%	82.89%
Chloroform extract of Licorice	400 mg/ kg	17.17±0.91*	74.25%	97.69%
Aspirin	100 mg/kg	16.00 ± 1.39*	76.0%	100%%

**Significant difference from control group at P< 0.05.*

Values given are means ± SE

Table: 5. Antinociceptive activity of some of different extracts of Glycerrhizaglabrata comparing with reference drug (morphine) using hot plate test

Animal Groups	Dose of drug	Reaction time (Sec) Mean± S.E	% Increase	% Analgesia
Normal Control	Saline	10.50 ± 0.34	-------	--------
Methanol extract of Licorice	400 mg/ kg	16.67 ± 0.61*	58.76%	57.83%
Chloroform extract of Licorice	400 mg/ kg	19.00 ± 0.37*	80.95%	79.66%
Morphine	10 mg/kg	20.67 ± 1.74*	96.85%	100%%

****Significant difference from control group at P< 0.05.***

Values given are means ± SE

DISCUSSION

Acute toxicity

Chloroform and methanol extracts of *Glycerrhiza* each was orally administered to mice in a dose of 0.1- 4.0 g/kg had no affect on their behavioral responses during the observation period of seven days after administration. No mortality was observed up to seven days of monitoring.

LD50 was found to be more than 4g/kg body weight orally. No morbidity or mortality was recordedfor the tested compounds. The therapeutic dose used in this study was 200mg/kg p.o. which was 20-fold less than the dose used in acute toxicity.

Parasitological results

Table 1 showed that infection with Schistosomamansoni recovered 25.67±1.65 worms, about one quarter of them present in the hepatic vessels (4.67±0.41) and the remainder present in the portomesenteric vessels (21.00±1.31). The total number of male and female worms nearly equal and the number of coupled worms was (7.67±0.56).

Treatment with chloroform extract of licorice gave the best results when compared with methanol extract of licorice. They increased the total number of hepatic worms equally to be (8.00±0.36) VS (8.5±0.22), while the total number of portomesenteric worms greatly reduced after treatment with chloroform extract of licorice to be (1.67±0.21) when compared with methanol extract of licorice (11.5±0.7). So, the total number of worms was reduced by 62.33 % VS 22.08% after treatment with chloroform extract and methanol extract respectively when compared to infected control group. Hepatic worm shift was more pronounced with chloroform extract. The total number of male and female worms nearly equal, but there was reduction in the total number of coupled worms

by 73.92% VS 28.29% after treatment with chloroform extract and methanol extract respectively when compared to infected control group. On the other hand, treatment with praziquantel (the drug of choice in the treatment of schistosomiasis) was found to reduce the total number of male and female worms to be 0.67±0.21 and 0.17±0.17 respectively. The total number of worms was reduced by 96.77% with complete eradication of coupled worms (Table 1). Table 2 showed that infection with *Schistosomamansoni* produced (38.5±2.55 X 10^3) ova; (11.78±1.02 X $10^{3)}$ ova present in liver and (26.72±2.36 X 10^3) in the intestine. The number of dead ova was about 12.53% and the rest of ova were equally divided as immature and mature ova. Treatment with chloroform extract was highly reduced the total number of *S. mansoni* eggs by about 62.41 % and the total number of dead ova was increased to be 19.32% and the total number of immature ova was reduced to be 31.08%. Treatment with methanol extract was highly reduced the total number of *S. mansoni* eggs by about 28.23 % and the total number of dead ova was increased to be16.38±5.77 and the total number of immature ova was reduced to be 38.17±5.28. Praziquantel reduced the total number of ova by 90.18% and all of them were dead.

Biochemical results

Table 3 showed that, infection with *Schistosomamansoni* for 9 weeks post infection increased the level of ALT and AST by about 108.9% & 119.7%. Treatment with chloroform extract of licorice gave more reduction in the level of ALT by about 35.8% and AST by about 29 % when compared with methanol extract of licorice (31.7% and 27.5%) respectively. Infection with *Schistosomamansoni*did not affect the serum level of protein but decrease the level of albumin. So, A/G ratio reduced than 1. Treatment with chloroform and methanol extract of licorice improved the level of albumin and so, it increase A/G ratio to be near normal value. Treatment with praziquantel improves all the biochemical parameters tested and tend to normalize.

Antinenoceptive activity

In acetic acid-induced writhing in mice, the total number of writhing produced along 20 after I.P injection of acetic acid was about 66.67 ± 1.91. More reduction in the number of writhing was recorded by chloroform extract of *Gylerrhiza*(74.25%) followed by methanol extract (62.99%) when compared to control group (Table 4). The % potency of chloroform extract was 97.69% followed by methanol extract 82.89 % when compared to aspirin.

In hot plate test, oral administration of chloroform extract increase in the latency time by 58.76% followed by methanol extract 85.71 % when compared to normal control group ($p<0.05$) (table 5). The % analgesia of chloroform and methanol extracts was found to be 60.67% and 83.57% respectively when compared to reference analgesic drug (morphine).

Tail immersion test, as demonstrated in Fig. (1), administration of chloroform and methanol extracts caused significant increase in the tail-flick response latency time as compared to control group ($p<0.05$) either after 60 or 120 min from drug administration. Chloroform extract showed more analgesic effect than methanol extracts when compared with morphine.

Phytochemical studies of methanol and chloroform extract:

Phytochemical tests were carried on methanol and chloroform extracts. It revealed the presence of Glycosides, saponins, triterpenes, phenolics and flavonoids compounds in methanol extracts. Chloroform extract showed presence of triterpenes, flavonoids and phenolic compounds. Chromatographic fractionation of both extracts using column and TLC chromatography give eleven compounds, five compounds were isolated from methanol extract and identified as

licochalcone B[19], isolicoflavanol[20], licorice saponin L[21], isoliquirtin**[21]** and licoagrochalcone D[22] and six comopounds were isolated from choloroform extract and identified as lupeol[23], P-coumaric acid [24], Kaempferol[25],quercetin[26], Protocatechuic acid[27], and cinnamic acid[28]. The nature of the isolated compounds from chloroform extract may be responsible for its high activity. All the isolated compounds are known compounds and were previously isolated from licorice and other plants. They were identified on the basis of spectroscopic analysis as mass spectrometry and nuclear magnetic resonance and comparable with published data and available authentic samples.

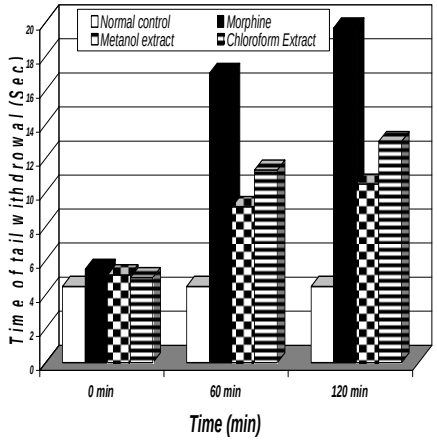

Fig. 1. Effect of chloroform and methanol extracts administered orally in a dose of 400mg/kg in the tail immersion test at 50 oC in mice compared to group treated with morphine in a dose of 10mg/kg. Time of tail withdrawl was calculated after 0, 60 and 120 min after administration of morphine and plant extracts. Each column represents the mean of 6 mice in each group.

CONCLUSION

In this study the methanol and chloroform extract of *Glycyrrhiza glabra* were evaluated for antischistosomal and antinenoceptive activities. Parasitological studies of plant extracts in *S.mansoni* infected mice showed that treatment with chloroform extract gave the best result when compared with methanol extract, Chloroform extract also showed more analgesic effect than methanol extract. Phytochemical analysis was carried on both extract to explain the activity of chloroform extract. Chromatographic isolation of the two extracts lead to isolation of eleven compounds.

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