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Complementary Effect of Neem and Mirazid on Mice Experimentally Infected with *S. mansoni*

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ABSTRACT

Aim*:* There is an increased demand for using plants in therapy "back to nature" instead of using synthetic drugs, which may have adverse effects that may be more dangerous than the disease itself. Mirazid *(Commiphora molmol*, MZ) and Neem (*Azadirachta indica*) are considered as a new safe and effective treatment of murine schistosomiasis, which has side degree of prevalence and spreading not only in Egypt, but worldwide. The current work has studied the complementary effect of Neem and Mirazid on mice experimentally infected with *S. mansoni*. ***Methods:*** Swiss albino mice were infected with 75 ±10 cercariae (obtained from Schistosome Biological Supply Centre (SBSC), Theodore Bilharz Research Institute (TBI), Imbaba, Giza; Cairo Egypt) and randomized into groups of 8 for neem extract (400 mg/kg) treated groups, positive control groups treated with conventional drugs PZQ (200 mg/kg) and MZ (500 mg/kg), as well as infected but untreated or treated but uninfected (negative control) groups. The study was done at the Animal Facility in the Department of Zoology, Faculty of Science, Al-Azhar University. ***Results:*** Both MZ and *A. indica* showed significant dose-dependent percentage worm load reduction ($P<0.001$). These extracts also significantly reduced tissues (liver and intestine) egg load counts. Infection with Schistosome increased systemic AST, urea and creatinine but decreased ALT and GGT.The different treatments used in this work did not significantly differ from positive controls except in case of treatment with neem extract where it decreased the creatinine level to reach normal values when it was used alone or complementary to Mirazid. ***Conclusion:*** The antischistosomal activity of the two plant extracts (MZ and neem) was dose dependent being more potent in reducing both the worm burden and tissue egg load. The antishistosomal effect of neem extract was significantly higher than Mirazid and mix of Mirazid with neem. These findings validate the potential use of neem extract in the management of schistosomiasis and provide a basis for exploring medicinal plants as sources for new antischistosomal agents.

Keywords: Neem, Mirazid, Mice, *S. mansoni*

Introduction

Schistosomiasis is prevalent in tropical and sub-tropical areas, especially in poor communities without access to safe drinking water and adequate sanitation. It is estimated that at least 90% of those requiring treatment for schistosomiasis (230 million people) live in Africa (WHO, 2012). Schistosomiasis is endemic in 74 countries worldwide with 20000 deaths associated with the severe consequences of infection (WHO, 2012). There are limited options available for the chemotherapeutic treatment of Schistosoma infection with the drug of choice being praziquantel (PZQ) (WHO, 1993, 2010).

Praziquantel has been the drug of choice for treatment of all species of schistosomes because of its efficacy, ease of administration, safety, and low cost (Cioli and Pica-mattoccia, 2003). However, PZQ is only effective against adult worms and ova of schistosomes but is ineffective on immature worms that are present in recently acquired infections and this leads to reduced cure rates (Gönnert and Andrews, 1977; El-lakkany *et al.,* 2011; Doenhoff *et al.,* 2008). In Egypt where there has been heavy exposure to PZQ, there are reports of *S. mansoni* and *S. haematobium* resistance to treatment (Utzinger *et al.,* 2003).The effectiveness of PZQ is dependent on an intact immune system (Ross *et al.,* 2002) and its mechanisms of action are poorly understood (Brindley *et al.,* 1989) . At the recommended dosage, PZQ achieves at best a 70- 90% worm reduction and its efficacy is lowest in heavily infected individuals (Raso *et al.,* 2004).

In the last decades, plant extracts were widely used for the treatment of Schistosoma infection (Sparg *et al.,* 2000; Molgaard *et al.,* 2001). Medicinal plants provide a rich source of biologically active

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compounds may offer alternative remedies in the management of schistosomiasis and indeed there are reports of several plant species with anti-Schistosoma properties (Sparg *et al.,* 2000; Molgaard *et al.,* 2001;Heinrich *et al.,* 2004; Dai and Mumper, 2011). In Egypt, the alcoholic extract of the oleo gum resin from Mirazid (myrrh, *C. molmol*) is used in treating schistosomiasis in experimental mice. Oral administration of the combination resulted in a significant reduction in the worm burden separation of females from males, and shifted the females from their normal habitat in the liver. Also there was marked reduction in the number of immature eggs (Badria *et al.,* 2001; Massoud *et al.,* 2005b). The safety and efficacy of *C. molmol* extract in treating heterophyiasis (100%), fascioliasis (100%) and schistosomiasis (92.5%) were documented (Massoud *et al.,* 2010). The resin extract of *C. molmol*as schistosomicide, fasciolicide, heterophycide and molluscicide have been reviewed in detail and provided sufficient evidence for its uses as antiparasitic agent (Tansatit *et al.,* 2012).

Neem (*Azadirachta indica*) has been known for more than 2000 years and has more than 100 common names. Today the Neem tree is widely distributed in the arid tropical and sub-tropical zones of Africa, Asia, Australia and the Americas (Ahmed and Ahmed, 2003). Neem of offers plenty of usages in several shapes and sizes. It has anti-fungal, anti-bacterial, anti-viral, and antidiabetic properties. It can treat various diseases and disorders ranging from malaria to bad teeth. Almost all parts of this tree contain medicinal benefits. Its seeds, leaves and barks have compounds known as limonoids with proven medicinal properties. Biologically, Neem has numerous bioactive ingredients with diverse applications. These bioactive ingredients are known to have antiallergenic, antidermatic, antifeedent, antiviral, antifungal, anti-inflammatory, antipyorrhoeic, antiscabic, insecticidal, parricidal, anti-implantation, nematicidal, and spermatocidal and other biological activities (Ogbuewu *et al.,* 2011; Reutemann and Ehrlich, 2008).

In the current study, a comparison between the effect of Neem extract *A. indica* (family: Meliaceae, genus: Azadirachta) and *Commiphora molmol* (family: Burseraceae) against adult worms of *S. mansoni* in infected mice has been investigated. As explained above, these plants have been reported to be used traditionally for treatment of parasitic infections and abdominal complications.

Materials and Methods

Infection of Experimental Mice with S. mansoni Cercariae:

Mice used in this study were Swiss albino, males, aged 6 weeks old and weighing 20-25 grams. Freshly shed cercariae (not more than 1 hour old) were used. Each mouse was infected with 75 ±10 cercariae subcutaneous in Theodore Bilharz Research Institute (TBI), Imbaba, Giza; Cairo Egypt. The infected mice were randomized into cages in groups of 8 and maintained with pellets food and water. The mice were allowed to adapt to the laboratory environment for one week before the experiment.

Drug Treatment of S. mansoni Infected Mice:

Praziquantel (PZQ, Discocide) Tablets: PZQ,is manufactured by Egyptian International Pharmaceutical Industries Company (EPICO). PZQ is known to target adult worms. Its chemical formula is 2- cyclohexylcarbonyl 1.2,3.6.7 11b hexahdro- 4h- pyrazino- (2,1-a) Isoquinoline-4-one. PZQ was given orally (for treatment at first day of 7 weeks p.i) in a dose of 200 mg/kg body weight per day using a dose volume of 0.05 ml for 5 consecutive days to a total dosage of 1000 mg/kg (Ahmed and Ahmed, 2003). The tablet was dissolved in 10% of dimethyl sulphoxide. Infected untreated negative control groups were also included that received 0.4 ml of dimethyl sulphoxide.

Mirazid was used in a dose of 500mg/kg and given orally at 4 week post infection (p.i) for 5 consecutive days. Mirazid is obtained as oleoresin extract in capsules produced by Pharco Pharmaceuticals Company, Alexandria, Egypt. The desired Concentration of extract was dissolved in dimethyl sulfoxide (DMSO, Sigma). The plant produces an oleo gum resin that contains 2-8% essential oil 23-40% resin, 40-60% gum and 10-25% bitter principles (El-Shahat *et al.,* 2011; Su *et al.,* 2011). Some active constituents of myrrh include the sesquiterpenes furanodiene-6-one and methoxyfuranoguaia-9-ene-8-one, which have antibacterial, antifungal and local anesthetic activities, (Dolara *et al.,* 2000) and furanoeudesma-, 3-diene and curzarene, which have potent analgesic effects (Dolara *et al.,* 1996). The furanosesquiterpenoids lindestrene and its analogues make up 19% of the essential oil and are responsible for the famous scent associated with myrrh (Jensen). Other important constituents are terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives which give myrrh its manifold biological activities (Su *et al.,* 2011).

Neem extract,*A. indica* (AZAL T/S (1%) Trifolio-MGmbH, Germany: Major chemical constituents of neem are Terpenes and Limonoids. The major active components in the Limonoids are azadirachtin, 3-deacetyl-3- cinnamoylazadirachtin, I-tigloyl-3-acetyl-II-methoxyazadirachtin, 22, 23-dihydro-23β-methoxyazadirachtin, nimbanal, 3-tigloylazadirachtol, 3-acetyl-salannoV nimbidioV margocin, margocinin, margocilin and others. Terpenoids are isoazadirolide, 6 nimbocinolide, nimbonone, nibonolone, methylgrevillate and Margosinone. Neem increases the production of Glutathione-s-transferase, thus improving the ability of the liver to detoxify itself of chemical contamination[6].Treatments with the plant extracts were done at 4 week post infection (p.i) to represent adult worm respectively. The plant extracts were administered at dose of 400 mg/kg. Plant extract was administered orally by gastric gavage once a day for 5 consecutive days using an oral volume of 0.4 ml *A. indica* per mouse (Ogbuewu, 2009).

Studied Groups:

All animals have been infected with 60 ±10 cercariae subcutaneous. Animals were divided into five groups: Group 1 is Neem treated group (Infected and treated with Neem extract orally in a dose of 400 mg/kg body weight for 5 consecutive days); Group2 is Neem and Mirazid treated group (Infected and treated with Neem and Mirazid extracts orally in a dose of 400 mg/kg and 500 mg/kg body weight respectively for 5 days); Group 3 is Mirazid treated group (Infected and treated with Mirazid orally in a dose of 500mg/kg body weight for 5days); Group 4 is Praziquantel treated group (Infected and treated with praziquantel orally in a dose of 200 mg/kg body weight for 5 days); Group 5 is Infected control mice and Group 6 isnormal mice. All the above infected groups had control treated but not infected groups. Animals were sacrificed at the 7^{th} and the 9^{th} weeks post infection.

Parasitological Criteria:

Worm load counts:

The groups of mice were sacrificed 3 weeks post treatment by injecting them with 0.25 ml of heparinized sodium pentobarbitone intraperitoneally to euthanize them and the worms recovered through perfusion of liver and mesenteric veins (Utzinger *et al.,* 2002). Liver and intestine were recovered from the perfused mice, wrapped in foil paper and stored in a freezer at -20ºC until used. Recovered worms were collected in petri-dishes and enumerated under a dissecting microscope.

Tissue egg load count:

The stored liver and guts were processed for tissue egg counts by trypsin digestion. Briefly, the number of ova/g hepatic or intestinal tissue was counted after digestion overnight in 5% KOH. Eggs were counted under a microscope using 10X magnification. A mean was calculated to estimate the number of eggs per ml. This was multiplied by the volume of egg suspension to derive the number of eggs in the whole tissue (Smithers and Terry, 1965).

Percentage egg developmental stages "oogram pattern":

The percentages of eggs at the developmental stages were examined in three samples/mouse and the mean of each stage/animal was obtained (Cheever, 1968).

Biochemical analysis:

Liver function:

The sera samples were subjected to liver and kidney function tests, (AST, ALT, GGT U/L, urea mg/dl and creatinine mg/dl).Colorimetric determination of alanine aminotransferase (ALTU/L) and aspartate aminotransferase (AST) was estimated, the color of which was measured at 546 nm according to Duvall and Dewitt (1967).γ Glutamyl transferase (γ GTU/L) was measured at 405nm according to Pellegrino *et al.*(1962).

Kidney function:

Urea (mg/dl) was measured by colorimetric method according to Abdul-Ghani *et al*. (2009). Creatinine (mg/dl) was done according to Gupta *et al*. (2008).

Statistical analysis:

Results were expressed as means ± standard error of the means (SE). Differences between groups were analyzed by using one way analysis of variance (ANOVA).The mean number of worms and eggs recovered from the different groups were subjected to Student's t-test using Microsoft Excel to determine their statistical significance in comparison with the control groups. The data were considered significant if $P < 0.05$.

Results

The comparison between the effect of Mirazid and Neem extracts has been achieved using parasitological and biochemical analysis. Worm load, tissue bound ova and oogram were used as parameters for parasitological study. Liver and kidney function tests were also used as biochemical parameters.

Worm load

The results obtained in the current study showed significant reduction ($P<0.001$) in the mean number of separate male and female worms in all the infected treated groups (9 weeks p.i) compared to the infected untreated group (G5). While the mean number of separate male and female worms in all the infected treated groups (7 weeks p.i) exhibited non-significant reduction in worm-load compared to the infected control group (G5). There was non significant reduction in the mean number of coupled worms in all treated group (7 and 9 weeks p.i). The total number of worms, both at 7 and 9 weeks p.i, recorded highly significant reduction

Table 1: Mean number of worms recovered from livers and intestines following treatment with Neem (*A. indica*) and Mirazid at 4 weeks (scarified 7 and 9 weeks) post infection.

Groups	Number of male worms (M ±SE)		Number of female worms (M ±SE)		Number of Copula worms (M ±SE)		Total number of worms (M ±SE)		% of protection	
	7w	9w	7w	9w	7w	9w	7w	9w	7w	9w
G1 Neem only (400 mg/kg)	2.3±3.2 acde	0.0±0.0 ***ce	1±1.73 acde	0.0±0.0 ***ce	7±2.6 ace	3.5±0.7 acde	11.3±5.5 ***	3±1 ***	75.9%	94.8 %
G2 Neem+Mirazid	4.3±6.6 abde	7±5.6 **de	0.3±0.5 *bde	7.5±4.9 ***d	15.6±12.7 abde	7.5±0.7 ad	20.3±5.5 ***	22±9.9 ***	56.8%	62%
G3 Mirazid only (500 mg/kg)	6.3±4.9 abce	2.5±3.5 ***ce	0.6±1.2 abce	3±2.8 ***c	14±9.1 abce	6.5±2.1 a	21±4.3 ***	12±4.2 ***	55.3%	79.3 %
G4 Praziquantel(200 mg/kg)	8±7.5 abcd	3±0.0 ***cd	0.5±1 *bcd	3±0.0 ***cd	7.25±8.2 ab	2.5±0.7 ad	15.7±15.5 **	7±1 ***	66.59%	87.9 %
G5 Infected only (control)	4±2.8	22±0.7	3±1.41	26±2.8	10±2.8	7±4.24	47±141	58±0.71	-------	------

(a) Non-significant difference from the G5 at P ≥0.05., (b) Non-significant difference fromG1 atP≥0.05.
(c) Non-significant difference from infected G2 at P≥0.05., (d) Non-significant difference fromG3 atP≥0.05.
(e) Non-significant difference fromG4 at P ≥0.05.,
*(***) Significantly different from control infected group at P ≤ 0.001 , ** at P≤ 0.01 and * at P≤0.05.*

The percentage of decrease in the total number of wormswas 94.8% at (9w p.i) and 75.9% at (7 weeks p.i) respectively in the mice that were treated at 4 weeks p.i at doses of 400 mg/kg using *A. indica* extract (Neem, G1). While the percentage of decrease was 87.9% at (9 weeks p.i.) and 66.59% at (7 weeks p.i) respectively, in the mice treated with PZQ after infection, group (G4). The percentage of decrease was

79.3% in Mirazid treated group (G3) while Neem and Mirazid treated group (G2) recorded a percentage of decrease as 62%, at 9 weeks p.i.

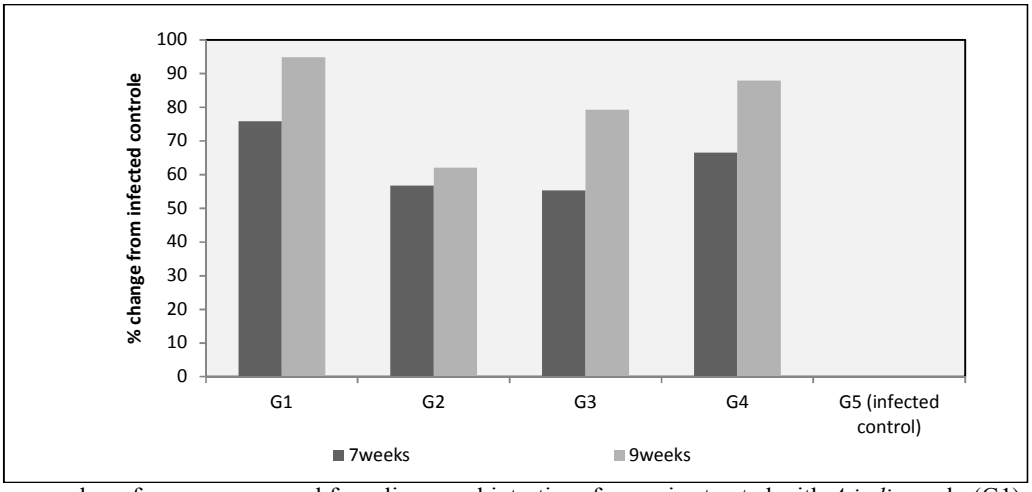

Fig. 1: Mean number of worms recovered from livers and intestines from mice treated with *A.indica* only (G1), Neem+Mirazid (G2), Mirazid (G3), PZQ (G4) and infected only (G5) at 4 weeks (scarified 7 weeks) post infection.

Tissue bound ova:

Regarding the ova count, the results obtained showed a highly significant (P≤0.001) reduction in the mean number of ova count/g liver between infected controls (G5) and all groups at 7weeks p.i. While there was no significant reduction in the mean number of ova count/gm liver in Neem and Mirazid treated (G2) and Mirazid treated groups (G3) at 9 weeks p.i., compared to (G5). While for the infected Neem treated (G1) and PZQ treated (G4), the reduction was highly significant. Also the results obtained showed a highly significant (P≤0.001) reduction in the mean number of ova count/gm intestine between infected controls (G5) and all groups at 7 weeks p.i. On the other hand only Neem treated (G1) the reduction was highly significant at 9 weeks p.i., compared to (G5),

Table 2: Mean number of eggs recovered from livers and intestines following treatment with *A. indica* and Mirazid at 4 weeks (scarified 7 and 9 weeks) post infection.

Groups	Number of eggs (livers) (Mean±SE)		Number of eggs (intestines) (Mean±SE)		% reduction in ova count (liver)		% reduction in ova count (intestine)	
	7w	9w	7w	9w	7w	9w	7w	9w
G1 Neem only (400 mg/kg)	1117.33±883.47 ***de	56±1.06 ***cd	1417.33±948.37 ***cde	96±0.71 ***	92.6%	95%	90.8%	91.8%
G2 Neem+Mirazid	2321.33±881.55 ***d	1166±308 ade	1153.33±779.51 ***bde	1536±893.78 ade	84.83%	-2.7%	92.5%	-31%
G3 Mirazid only (500 mg/kg)	870.67±608.82 ***be	808±593.97 abc	725.33±260.13 ***bce	1930±444.o6 *c	94.31%	28.8%	95.3%	-64%
G4 Praziquantel (200 mg/kg)	203±69.16 ***bd	12.5±0.71 ***cd	225.20±192.24 ***bc	657.80±0.28 ***c	98.6%	98.8%	98.5%	43.8%
G5 Infected only	15305.6±4.16	1135±1.41	15572±2.83	1172.5±3.54	---	---	----	----

(a)Non-significant difference from the G5 at P ≥ 0.05., (b) Non-significant difference fromG1 atP≥0.05.
(c) on-significant difference fromG2 at P≥ 0.05., (d) Non-significant difference fromG3 atP≥0.05.
(e) Non-significant difference fromG4 atP≥0.05.
(***) Significantly different from G5 at P≤ 0.001 ,(**) at P≤ 0.01 and (*) at P≤0.05.

The percentage of reduction in the mean number of ova count/g liver was 92.6% (G1), 84.8% (G2), 94.3% (G3) and 98.6% (G4) at 7weeks p.i.in comparison with group (G5).The percentage of reduction was 95%, -2.7%, 28.8%, and 98.8% respectively at 9 weeks post infection. The percentage of reduction in the

mean number of ova count/g intestine was90.8% (G1), 92.5% (G2), 95.3% (G3), and 98.5% (G4) at 7 weeks p.i. While the percentage of reduction in the mean number of ova count/g intestine was 91.8%, -31%, -64%, and 43.8% respectively at 9 weeks post infection

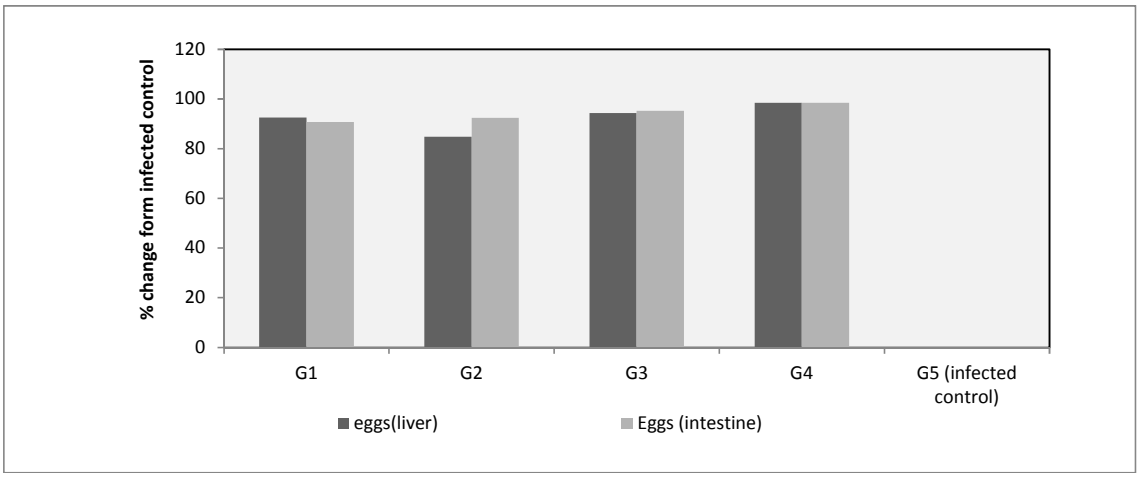

Fig. 2: Mean number of eggs recovered from livers and intestines following treatment with *A. indica* only (G1), Neem+ Mirazid (G2), Mirazid (G3), PZQ (G4) and infected only (G5) at 4 weeks (scarified 7 and 9 weeks) post infection.

Oogram study:

The resulted data revealed positive highly significant correlation only in Neem treated group (G1) in the mean number of immature, mature and dead egg in intestinal tissues (P≤0.001) at 9 weeks p. i. Other groups had non-significant correlation P≥0.05 except PZQ treated group had recorded a highly significant in the mean number of mature and dead egg (P≤0.001) in comparison with group (G5) at 9 weeks p. i.

Table 3: Mean number of developing eggs recovered from intestines following treatment with *A. indica* and Mirazid at 4 weeks (scarified 7 and 9 weeks) post infection.

Groups	immature Stage I		II		III		IV		Mature		Dead	
	7w	9w	7w	9w	7w	9w	7w	9w	7w	9w	7w	9w
G1 Neem only 400 mg/kg	15.8±8.2	58.3±13.3 ***	11.5±8.3	36±1 ***	5.7±4.2	47.3±1.5 ***	5.6±4.2	38.3±1.5 ***	46.3±43.6	91.3±1.5 ***	20.1±15.4	48±2 ***
G2 Neem+Mirazid	3.3±2.8	62.6±52.5	16.6±9.6 *	81±61.51	13.2±10.4	60±25 *	13.3±9.6	44.3±29.5	33.1±30.6	113±88	13.5±14.6	59±29 *
G3 Mirazid only 500 mg/kg	16.3±12.5	26.6±20.6	21.3±14.5	17±2 ***	17.8±16.9	35.3±9.5 *	3.2±4.7	36±18.5	67±64.8	73±28 *	25±22.05	46.6±28.5 **
G4 Praziquantel 200 mg/kg	3.5±3.11	26±9.6	8.2±6.2	13.6±8.5 *	2.8±3.6	10±10	0.5±1	22.6±17.5	101.5±56.6	36.3±27.5 ***	48.5±20.5	37.6±18.5 ***
G5 Infected only	5±7.07	23.5±2.1	2.5±3.5	26±1.41	8.6±10.2	19±1.4	10.6±9.2	19±2.8	37.3±54.6	117±4.2	27.6±26.5	98±2.8

(***) Significantly different from control infected group at P≤ 0.001(**) at P≤ 0.01 (*) and at P≤0.05.
Data are expressed as mean±SD, n=3 or 4.

Biochemical analysis:

Levels of AST in serum of all groups recorded a highly significant increase (P≤0.001) compared to normal (N) and infected control group (G5). Meanwhile liver ALT in all groups was significantly decreased by 20.6% G3, 41.7% G4 and 30.7% G2.However, Neem treated group (G1) recorded nonsignificant increase (P>0.05). Also, Levels of GGT in serum of all infected and treated groups recorded significant or a highly significant decrease (P≤0.01; P≤0.001) compared to normal and infected control groups(Table 4 and Fig 4).. Urea levels in serum of most groups recorded a highly significant increase (P≤0.001) except for PZQ treated (G4) group which recorded non-significant increase compared to normal control group. Creatinine levels in serum of all groups were significant increased, except for Neem treated (G1) group,

compared to normal control group (N). While all groups recorded a highly significant decrease compared to infected control group (G5) (P>0.001).

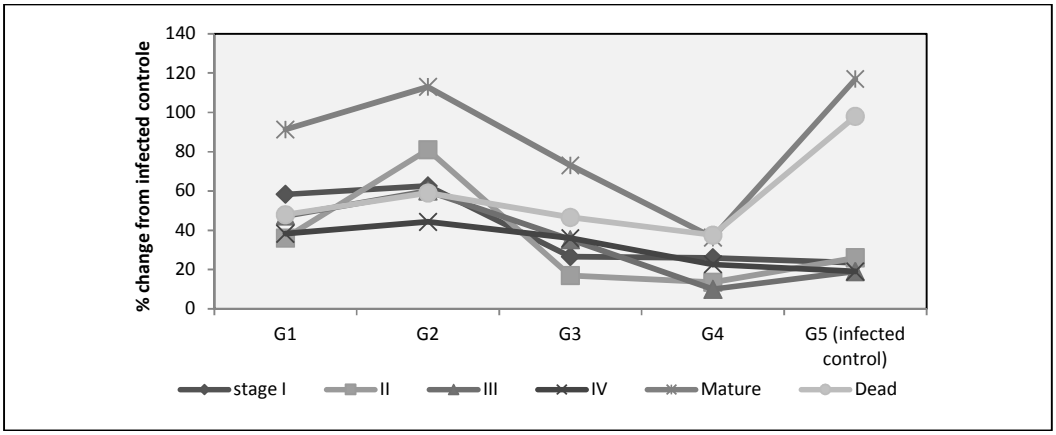

Fig. 3: Mean number of developmental stages of eggs recovered from intestines from mice treated with *A. indica* only (G1), Neem+Mirazid (G2), Mirazid (G3), PZQ(G4)and infected only (G5) at 4 weeks (scarified 7 weeks) post infection.

Table 4: Effect of Neem, Mirazid and praziquantel on liver and kidney functions in different groups at 7 weeks.

	ASTU/L		ALTU/L		GGTU/L		Ureamg/dl		Creatininemg/ dl	
	Normal N	Infected Control G5	Normal N	Infected ControlG5	Normal N	Infected Control G5	Normal N	Infected Control G5	Normal N	Infected Control G5
	45±0.58	126.6±8.82	46.7±2.25	24.5±4.9	21.5±3.5	7.31±2.23	76.75±1.75	120.6±8.51	0.48±0.03	1.63±0.40
		***		**		**		***		*
C4	200± 5.7		27.9 ±8.05		17.5±0.2		123.3±12.5		0.78± 0.11	
DMSO control	***	***		**	*					
G1	390± 5.77		55.7±4.5		11.48±8.3		104.53±8.75		0.45±0.13	
Neem	***	***					**			***
G3	226.6± 8.82		20.66±5.4		4.46± 1.5		128.92±8.99		1.22±0.49	
Mirazid	***	***	**		**		***			
G4	88.3± 6.01		41.76± 9.5		7.87± 4.3		99.39± 11.6		0.76± 0.29	
PZQ	***	***			*					
G2	305± 5.15		30.71±9.13		12.86±10.9		109.56±5.84		0.83±0.30	
Neem+Mirazid		***				***				***

** Significant difference from the control at P≤0.05, ** at P≤ 0.01 and *** at P≤ 0.001*
Data are expressed as mean±SD

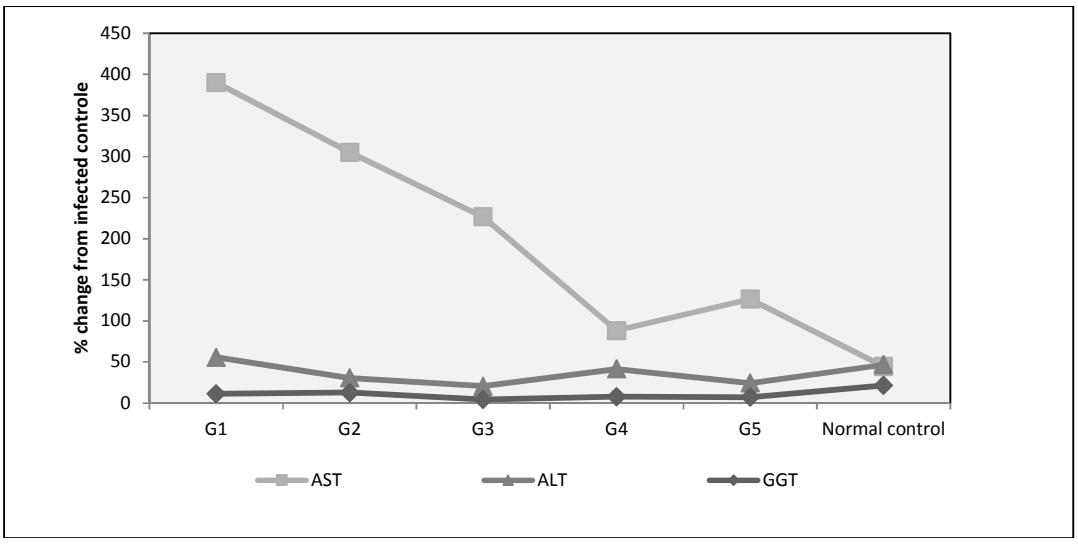

Fig. 4: Effect of Neem and Mirazid on Liver function of infected mice with *S.mansoni*from mice treated with *A. indica* only (G1), Neem+Mirazid (G2), Mirazid (G3), PZQ (G4) and infected only (G5) (scarified 7 weeks) post infection.

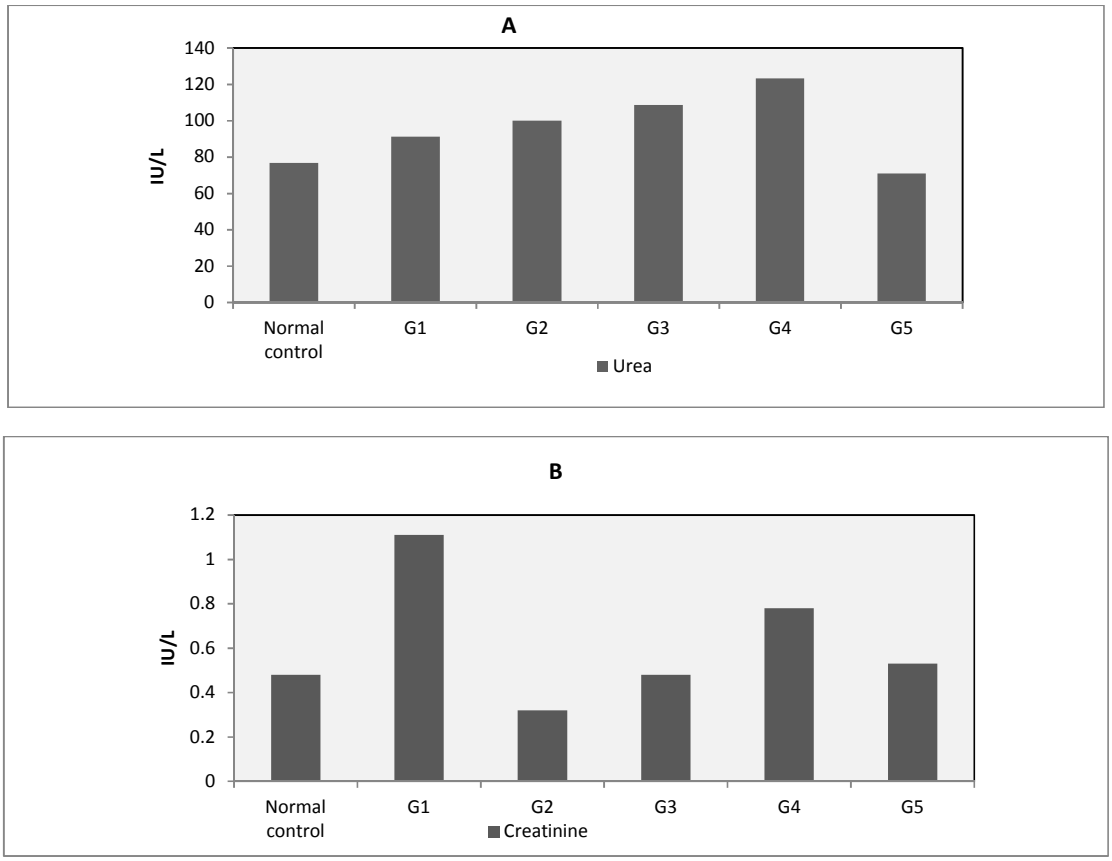

Fig. 5: Effect of Neem and Mirazid on kidney function of infected mice with *S. mansoni* from mice treated with *A. indica* only (G1), Neem + Mirazid (G2), Mirazid (G3), PZQ(G4)and infected only (G5) (scarified 7 weeks)post infection.

Discussion

Phytochemicals present in Mirazid had shown a series of metabolites including terpenoids, steroids, flavonoids, lignans, carbohydrates, and long chain aliphatic alcohol derivatives isolated and identified from Commiphora species. These secondary metabolites of the Commiphora species exhibited diverse biological activities, such ascytotoxic effects (Chattopadhyay *et al.,* 2000). The Neem (*A. indica*) leaf extract provided a hepatoprotective effect against paracetamol-induced hepatic cell damage in rats, findings supported by histopathological studies (Ogbuewu *et al.,* 2010). Administration of the mature leaf extract decreased serum cholesterol significantly without changing serum protein, blood urea and uric acid levels in rats (Ogbuewu *et al*., 2011; Reutemann and Ehrlich, 2008; Ogbuewu *et al.,* 2010b). Currently there are few drugs available for the treatment of schistosomiasis these being limited to PZQ which is the drug of choice as it is effective against all human infecting species of schistosomiasis and has the ability to irreversibly cause damage to adult worms and eggs lodged in host organ (WHO, (1993, 2010) ; Giboda and Smith, 1994). Nevertheless, PZQ has its limitations in that: it is less effective against developing worms necessitating multiple drug treatments (Utzinger *et al.,* 2003) ; at the recommended dosage it achieves at best a 70% - 90% worm reduction and its efficacy is lowest in heavily infected individuals (Raso *et al.,* 2004) ; there are reported cases of schistosome isolates with reduced susceptibility to PZQ in the field in endemic areas and under laboratory conditions (Raso *et al.,* 2004 ; You-Sheng *et al.,* 2001) which could see emergence and spread of PZQ resistant strains.

The results of the current study demonstrated that oral ingestion of Mirazid and *A. indica*plant extracts or their combination to infected mice was effective in reducing worm burden and egg count when compared with infected untreated mice, indicating their effective antischistosomal action. Reduction of worm burden and egg count with *A. indica* extractwas the highest percentage compared with Mirazid extract or their combination and also with PZQ (Tansatit *et al.,* 2012)]. Some authors demonstrated that the death of the worms due to the treatment with antischistosomal drugs was attributed to metabolic disorders, mechanical destruction and muscular contraction of treated worms (Doenhoff *et al.,* 2002; Ibrahim *et al.,* 2010; Emam *et al*., 2009) . The data is coincided with data obtained by Emam *et al*. (2009)and Kadry *et al*.(2013). Previous research works showed that using Mirazid as an adjuvant to the garlic and PZQ decreases their antibilharzial efficacy (El-Kott *et al.,* 2011; Riad *et al.,* 2012) . The current data have also shown that Mirazid decreases the antischistosomal efficacy of neem so that there is a need to identify the concentrations of the agents in the combination that are optimal in terms of both tolerability and therapeutic efficacy.

These current research results are in harmony with Musili *et al*. (2015)**.**There are no documented reports, before their work, of evaluation of (*A. indica*) extract for their potential anti-schistosome properties against adult worms. They demonstrated that (*A. indica) has* appreciable antischistosomal activity. It however showed a high activity on juvenile worms. A reduction in egg load could be due to induction of separation of males and females which in turn reduces or even arrests the release of eggs, which is a relevant factor in the hepatic pathology and the transmission of the disease. A reduction in worm-load especially in female worms results in a reduction in egg load in the tissues. Plant extracts with such activity have the potential of being used as transmission control tools and for intervention to ameliorate adverse effects due to disease pathology. The antischistosomal effects of these extracts on juvenile worms indicate that plant extracts may be used as complimentary drugs or in combination with conventional drugs such as PZQ or Mirazid for effective management of the disease (Tansatit *et al.,* 2012; Musili1 *et al.,* 2015)**.**

Similar results have been recorded on the effect of other plants against schistosome at different stages cercariae, schistosomula and adult worms [14]. Schistosomicidal activity of crudeaqueous extract of ginger against *S. mansoni* reported by Mostafa *et al.*(2011)] who observed that parasite load and egg density in the liver and feces of mice treated with ginger were less than their counterparts. Male worms recoveredfrom mice treated with ginger lost their normal surface architecture, extended erosion beyond the tegument, besides numerous bubbles around tubers. Mahmoud *et al*. (2002)reported that treatment of mice infected with *S. mansoni* parasite using black seed oil, was effective in reducing egg count in both liver and intestine.

Several factors may explain such reduction in schistosomal egg count. These factors are a probable diminished fecundity of the worm pairs and an increased rate of egg excretion due to the egg death. Teguments as well as the gut epithelium are two sites attacked by the drug (Nosseir *et al.,* 2000). It seems likely that the tegmental changes in the worms may be an important aspect of drug activity leading to the death and elimination of worms with stopping of their egg production (Nosseir *et al.,* 2000). Sharma and Agarwal (1996) stated that Cellular damage arises when the equilibrium between the amounts of ROS produced and that of scavenged antioxidants is disturbed. This data suggested that the used drug may lead to the impairment of ROS catabolizing system of the parasite, and such process is may be closely related to the killing of the parasite. Praziquantel, meanwhile, works by causing sever spasms and paralysis of the worm's muscles. This paralysis is accompanied-and probably caused-by a rapid influx of Ca^{2+} inside the schistosome (Doenhoff *et al*., 2008).

There was no significant difference between effect of the infection with *S. mansoni* and effect of the different treatments, used in this research work, on the liver and kidney functions of experimental mice except in case of using neem treatments where it decreases the creatinine level to reach the normal values either when it was used alone or combined with Mirazid. Elevated blood urea and serum creatinine were normalized after treatment (Hamed and Hetta, 2005).

In consistent with some studies (Mahmoud *et al.,* 2006), the present study showed that the inflammatory reactions induced in livers of *S. mansoni* infected mice are ensured by marked increase in serum AST. The increase of such enzymes in serum may be due to the destruction of hepatocytes by the action of toxins of the parasite eggs leading to their release into the circulation (Emam *et al.,* 2009; Miraglia *et al.,* 1981) this attributed to greater destructive changes in liver compared with other groups.

In agreement to the data obtained in this research, other researchers found that ALT and GGT levels are ensured by marked decrease (Ibrahim *et al.,* 2010; El-Shenawy and Soliman, 2003; Mohamed *et al.,* 2008; Morais *et al.,* 2010).However (Morais, 2005) in Egypt investigated the effect of *Schistosoma*

mansoni infection on mice livers after treatment with (Mirazid), as a new anti-schistosomal drug. Liver function enzymes; ALT showed significant drop after treatment, but still higher than normal.Dkhil, (2015) proved that the levels of the activates of the liver enzymes in the blood serum were significantly decreased in the liver homogenate of the infected mice due to the existence of the inflammatory hepatic granuloma reported to be present as a result of egg deposition and the presence of worms and their toxins. The decrease in serum may be due to reduction in its synthesis by damaged liver (Mahmoud *et al.,* 2006).

Similar to this study, other studies have shown that urea and creatinine increased in schistosomiasis (Prastowo *et al.,* 2014; El Shenawy *et al.,* 2008)**.** Schistosomal infection increases creatinine level due to immune complex deposition in the glomeruli resulting in true glomerulonephritis (Andrade and Abreu, 1971).

It could be concluded that *A.indica* extract could be used as antischistosomal drug. This study is the first to use the combination of Neem and Mirazid in schistosomal treatment in mice. Neem could be used as complimentary drug or in combination with conventional drugs such as PZQ or Mirazid for effective management of the disease. However, in this study treatment with only Neem extract showed high efficacy than other combination treatment. There should be an identification of the concentrations of the agents in the combination with Neem that are optimal for the treatment of schistosomiasis.

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