Journal of the Egyptian Society of Parasitology, Vol.52, No.2, August 2022
J. Egypt. Soc. Parasitol. (JESP), 52(2), 2022: 331 – 340
(Online: 2090-2549)

HEPATOPROTECTIVE EFFECT OF *UNCARIA TOMENTOSA* ON *SCHISTOSOMA MANSONI* EXPERIMENTAL INFECTION THROUGH REDUCTION OF NUCLEAR FACTOR KAPPA B

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Abstract

Schistosomiasis is a serious and neglected tropical illness with a negative influence on human health. It's treated with praziquantel (PZQ), which has been linked to resistance and reinfections. As a result, new anti-schistosomal medications are desperately needed. Medicinal plants, particularly crude preparations of medicinal herbs, have a potential treatment against schistosomiasis. *Uncaria (U.) tomentosa* is a rainforest plant native to the Amazon and Central America. It is commonly used in traditional medicine due to its immunomodulatory and anti-inflammatory effects. This study aimed to compare both antiparasitic and hepatoprotective efficacy of *U. tomentosa* bark extract to PZQ in mice infected with *Schistosoma mansoni*. To evaluate its antiparasitic qualities, worm load, egg count, granuloma counts, and diameters were measured. Also, liver tissues were immune stained using nuclear factor kappa-B (NF-B), and the levels of the inflammatory proteins interleukin (IL)-4, IL-5, and nitric oxide (NO) in the serum were assessed. *U. tomentosa* had a substantial impact on granuloma diameter but without significant impact on worm load, tissues egg load, or granuloma numbers. It has a good hepatoprotective effects through improvement of liver enzymes and reduction of NF-κB.

Keywords: *Uncaria tomentosa, Schistosoma mansoni*, nuclear factor kappa B

Introduction

Schistosomiasis is endemic in 55 nations with the worst regions were Africa, the Middle East, the Caribbean, Brazil, Venezuela, and Suriname (Silva-Moraes *et al,* 2019). After malaria, it is the second leading cause of death from parasites (Hotez and Fenwick, 2009; Keitel *et al,* 2019). Consequently, schistosomes affects 190 million individuals, with 70 million new infections annually (Global Burden of Disease Study, 2017), caused 21,151deaths in sub-Saharan Africa out of 24,068 global ones (WHO, 2018). It accounted to 4.5 million years of with life disability (Hailegebrie *et al,* 2021), with African Patients at least 90% need treatment (WHO, 2020). It affects liver causing granuloma formation with severe fibrosis that disrupts blood flow with subsequent portal hypertension that may lead to fatal esophageal bleeding. Hepatosplenomegaly and liver cirrhosis are commonly associated (Manzella *et al,* 2008).

Schistosomiasis treatment relies mainly on praziquantel (PZQ) which the known medication for decades. Despite reports of development of treatment resistance with rate low of cure and/or egg reduction (Fukushige *et al,* 2021). This resistance might be occurred due to its prolonged use either in cases of treatment or during mass drug administration (Ghazy *et al,* 2021). It is also associated with high rate of reinfection and high costs to cover all parts of an endemic area (Stephenson *et al,* 2014). These causes reinforced the need to for new safe, cheap and effective therapies against schistosomiasis.

The Egyptian Herbal extracts are considered the major source of biologically active compounds for new therapies against parasitic infections (Abdel Hady *et al,* 2008).

Uncaria tomentosa (*U. tomentosa*) *is* commonly known as cat's claw, originated from the Amazon rainforest and others of South and Central America as a tropical plant. As to toxicity outcome, rats given dosages of 8g/kg & 1g/kg/28days respectively, didn't show acute or chronic toxicity. More than 50

chemical components in total, including numerous secondary metabolites contained tetracyclic and pentacyclic oxindole alkaloids with anti-inflammatory, antioxidant, antineoplastic, antibacterial, antiprotozoal, and immuno-stimulant properties (Abou-el-Nour *et al,* 2015; Santos *et al,* 2016; Batiha *et al,* 2020). *U. tomentosa* immunological activity was mainly by immunological polarization to CD4 Th2 cells and their cytokines (Domingues *et al,* 2011b). It has hepato-protective properties by inhibiting nuclear fact- or kappa B (NF-B) that activate a number of pro-inflammatory and cytotoxic cytokines (TNF- & IL-6) that result in oxidative stress and liver damage. Also, the extract inhibits nitric oxide (NO) generation that decreased cytotoxic effect and hepatic inflammatory pathway, with down-regulating the expression of inducible nitric oxide synthase (Elgawish *et al,* 2019).

NF-κB (The nuclear factor-kappa B, p65) transcription factor is a main regulator of inflammation; whose activation is essential for release of pro-inflammatory cytokines from tissues exposed to pathogen-associated molecular patterns. It has a dual function in inflammation, acting both to cause and to resolve inflammation (Abd El-Aal *et al,* 2017). The NF-kB signals have a critical role in hep-atic fibrosis development by altering hepatocyte, hepatic stellate cells and Kupffer cell functions (Lawrence and Fong, 2010).

This study aimed to evaluate the antiparasitic and/or hepatoprotective effects of *U. tomentosa* bark extract on mice experimentally infected with *Schistosoma mansoni* by parasitological, histopathological, and immunological studies.

Materials and Methods

Ethics Statement: For the study, pathogen-free male BALB/c mice (6-8 weeks old, 18-20gm) were used. They were housed in a breeding environment in Theodor Bilharz Research Institute's (TBRI) animal house in Giza. Mice were fed a commercial pelleted diet in a breeding chamber kept at 20-22°C. All procedures were carried out in compliance with international ethical guidelines approved by TBRI's institutional ethical committee that agreed with Helsinki 92000)

Study Design: Mice were divided into five groups of ten mice each. GI: mice were non-infected and non-treated (negative control). GII: mice were infected with *S. mansoni*, but not received therapy (positive control). GIII: mice were infected with *S. mansoni* and treated with *U. tomentosa* bark extract. GIV: mice were infected with *S. mansoni* and subsequently treated with PZQ. GV: mice were treated by *U. tomentosa* bark extract & PZQ. Mice were infected with an Egyptian strain of *S. mansoni* by subcutaneous injection of 80-100 cercariae (Peters and Warren, 1969).

U. tomentosa bark extract & PZQ treatment: Extract was purchased from Creative Enzymes, USA. A 400 mg/kg oral dosage was administered to mice of GIII & GV strains for 21 days (Domingues *et al,* 2011a) from the day 30 post infection (PI). Praziquantel (Egyptian International Pharmaceutical Industries Company (E.I.P.C.O.) was suspended in 2% cremophore (Sigma Aldriich, USA) and given orally for two days at a dose of 500mg/kg 45 days PI. to GIV & GV (El-Lakkany *et al,* 2012).

Euthanizing mice and sample collection: At the end of the 8th week of PI, all mice were decapitated. Centrifugation at 3000rpm for 5 minutes was used to separate sera from peripheral blood, which was kept at -20°C until use. Livers were taken out and rinsed with physiological saline after worm counting.

Worm count: It was made by normal saline perfusion of hepatic and porto-mesenteric vessels through cannulation of inferior vena cava of euthanized mice. Male, female and coupled worms were counted (Duvall and DeWitt, 1967).

Tissue egg load: Liver and ileum samples were collected from each mouse, weighed, and digested in 5% KOH at 37°C for 16hr, at x40, ova were counted (Herbert *et al,* 2010).

H & E stain for bilharzial granuloma: Liver samples were fixed in formalin, paraffinized and stained. Granulomas number was

counted, and their diameters were digitally measured using an Olympus SC100 multihead microscope. Only the diameter of granuloma with a single ovum was considered.

Immunohistochemical staining: Immune staining of paraffinized liver sections was done using nuclear factor kappa-B p65 (NF-κB p65) (Cell Signaling Technology, USA) after Ashour *et al.* (2015). Positivity was confirmed by brown cytoplasmic and nuclear staining. Stained slides were evaluated quantitatively using the H-score. Briefly, intensity of staining was assigned a number ranging from (1^+, 2^+ & 3^+) for mild, moderate, and strong staining respectively. Percentage of stained cells in each tissue was multiplied by its intensity. A score of 0-300 was given to staining (Fraser *et al,* 2003), bio-marker after following equation H score = 1 × (% ce lls 1^+) + 2 × (% cells 2^+) + 3 × (% cells 3^+).

Assessment of IL-4, IL-5 &NO serum levels: Serum levels of IL-4, IL-5 and NO were measured by a solid-phase sandwich ELISA. IL-4 & IL-5 Kits were purchased from Thermo-Fisher Scientific, USA. NO level was measured using commercial kit (Bio-diagnostic Co., Egypt). Steps were carried out in accordance with the manufacturer's methodology. OD values were measured at 450nm absorbance except serum NO level at 550nm with an ELISA reader (Bio-Rad, UK).

Liver enzymes: As a biomarker of liver injury, ALT and AST were measured. They were calorimetrically estimated using standa rd diagnostic kits (BioSystems S.A. Costa Brava, Barcelona (Spain) after Reitman and Frankel (1957).

Statistical analysis: Data were collected, tabulated, and analyzed using an IBM personal computer with Statistical Package of Social Science (SPSS) version 20 (IBM Corporations, 2011), Armonk, NY and Epi Info 2000 programs, where the following statistics were applied. Descriptive statistics: Quantitative data were presented in form of mean ($\overline{X}$), standard deviation (SD), range, and qualitative data were presented in numbers and percentages (%). Analytical statistics: One-way ANOVA test (F) is a test used for comparison between more than two groups having quantitative parametric variables. Student's t- test is a test used for comparison between two groups having quantitative parametric variables used for post hoc test. P-value of >0.05 was considered not significant, P-value of ≤0.05 was considered statistically significant and P-value of ≤0.001 was considered highly significant.

Results

The highest male worm load reduction was in mice treated with PZQ, without significant difference between groups received PZQ alone (69.05%) and those received *U. tomentosa*+ PZQ (66.7% or P6 >0.05). Female worm load reduction percent was (80.9%, & 88.1%, respectively) and coupled worm load yielded similar findings (79.7%, & 88.4% respectively). When compared *U. tomentosa* treated group to infected non-treated one, no significant difference was found (P1: >0.05).

As regards to tissue egg load-either that of intestine or liver, highest reduction was recorded in both mice groups that treated with PZQ without significant difference between those who received PZQ alone (88.8% intestine & 80.7% liver) or treated with *U. tomentosa* + PZQ (88.8% intestine & 80.5% liver) (P6: >0.05). *U. tomentosa* alone showed a significant reduction of intestinal and liver egg load (P1: <0.001) when compared with infected non-treated one, but with significantly reduction than induced by PZQ.

Granuloma number, highest reduction was detected in PZQ treated groups either alone (51.6%) or treated with *U. tomentosa* + PZQ (73.6%) without significant difference between them (P6: > 0.05). Reduction in mice treated with *U. tomentosa* alone was 41.8%, with a significant difference when compared with infected non-treated ones (P1: <0.001). The opposite was observed in granuloma diameter, where *U. tomentosa* showed the best results. Highest reduction was found in the *U. tomentosa* treated groups either alone (80.2%) or with PZQ (82.1%), but without significant difference (P5: >0.05). PZQ treated group showed a significant lower diameter reduction (41.9%) than both *U. tomentosa* treated ones (P4 & P6: <0.001).

As to immunohistochemical staining of liver tissues, lowest mean H-scores of NF-κB was recorded in *U. tomentosa* treated mice either alone (22.3±1.8) or with PZQ (21.1± 1.79), but without significant difference (P5: >0.05). PZQ treated group showed highest score (36.1±2.07) with a significant difference between them and *U. tomentosa* treated groups (P4 & P6: <0.001). Highest mean serum level of IL-4 & IL-5 was detected in *U. tomentosa* treated onesalone (100±2.7, 94.8±2.4 respectively) or with PZQ (104.1± 5.9, 96.6±1.5), but without significant difference between both groups (P5: >0.05). PZQ treated mice showed lowest serum level (72.9±5.8, & 65±3.9 respectively) with a significant difference between them and *U. tomentosa* treated ones (P4 & P6: <0.001). Reverse was in NO serum level, both *U. tomentosa* treated ones gave lowest mean serum level either alone (8.6±-0.46) or with PZQ (8.3±0.28), but, without significant difference between both groups (P5: >0.05). PZQ treated group showed hig-hest mean serum level in all treated groups (10.9±0.53) with a significant difference between them and *U. tomentosa* treated ones (P4 & P6: <0.001). Best improvement of liver enzymes ALT & AST was detected in both *U. tomentosa* treated groups either alone (70.2% & 64.1% respectively) or with PZQ (71.6% & 66% respectively), but without significant difference between both groups (P5: >0.05)

Details were given in tables (1, 2, 3 & 4) and figures (1, 2, 3, 4 & 5).

Table 1: Comparison between mean worm loads in groups:

Variations		No.	$\bar{x}$ ± SD	% of reduction	ANOVA (F test)	Post Hoc test
Male worms	Infected	10	4.2±0.79		P<0.001**	P1: >0.05
	U. tomentosa	10	3.9±0.88	7.1%		P2: <0.001**
	PZQ	10	1.4±0.52	66.7%		P3: <0.001**
	U. tomentosa + PZQ	10	1.3±1.25	69.05%		P4: <0.001**
						P5: <0.001**
						P6: >0.05
Female worms	Infected	10	4.2±0.79		P<0.001**	P1: >0.05
	U. tomentosa	10	3.8±0.79	9.5%		P2: <0.001**
	PZQ	10	0.80±0.79	80.9%		P3: <0.001**
	U. tomentosa + PZQ	10	0.50±0.54	88.1%		P4: <0.001**
						P5: <0.001**
						P6: >0.05
Couple worms	Infected	10	6.9±0.74			P1: >0.05
	U. tomentosa	10	6.3± 1.1	8.6 %		P2: <0.001**
	PZQ	10	1.4±1.26	79.7%		P3: <0.001**
	U. tomentosa + PZQ	10	0.80±0.92	88.4%		P4: <0.001**
						P5: <0.001**
						P6: >0.05

Comparison; P1: between infected mice & *U. tomentosa* treated ones. P2: between infected mice & PZQ ones. P3: between infected group & *U. tomentosa* + PZQ ones. P4: between *U. tomentosa* mice & PZQ ones. P5: between *U. tomentosa* mice & *U. tomentosa* + PZQ ones. P6: between PZQ mice & *U. tomentosa* + PZQ.

Table 2: Comparison between mean tissue egg loads in groups:

Variations		No.	$\bar{x}$ ± SD	% of reduction	ANOVA (F test)	Post Hoc test
Intestinal Egg Count	Infected	10	7381±159.8		P<0.001**	P1: <0.001**
	U. tomentosa	10	6250±161.5	15.3%		P2: <0.001**
	PZQ	10	830±18.4	88.8%		P3: <0.001**
	U. tomentosa + PZQ	10	829.4±15.4	88.8%		P4: <0.001**
						P5: <0.001**
						P6: >0.05
Liver Egg Count	Infected	10	3731.7±160.2		P<0.001**	P1: <0.001**
	U. tomentosa	10	1363±83.6	63.4%		P2: <0.001**
	PZQ	10	721±14.9	80.7%		P3: <0.001**
	U. tomentosa + PZQ	10	728.5±14.7	80.5%		P4: <0.001**
						P5: <0.001**
						P6: >0.05

Table 3: Comparison between mean numbers and diameters of liver granulomas in groups.

		N	$\bar{x}$ ± SD	% of reduction	ANOVA (F test)	Post Hoc test
Granuloma number	Infected	10	9.1±0.88		P<0.001**	P1: <0.001**
	U. tomentosa	10	5.3±0.67	41.8%		P2: <0.001**
	PZQ	10	4.4±0.52	51.6%		P3: <0.001**
	U. tomentosa + PZQ	10	4.0±0.47	73.6%		P4: <0.05*
						P5: <0.001**
						P6: >0.05
Granuloma diameter	Infected	10	227.3±13.8		P<0.001**	P1: <0.001**
	U. tomentosa	10	44.9±5.4	80.2%		P2: <0.001**
	PZQ	10	132±9.2	41.9%		P3: <0.001**
	U. tomentosa + PZQ	10	40.6±6.8	82.1%		P4: <0.001**
						P5: >0.05
						P6: <0.001**

Comparison; P1: between infected mice & *U. tomentosa* treated ones. P2: between infected mice & PZQ ones. P3: between infected group & *U. tomentosa* + PZQ ones. P4: between *U. tomentosa* mice & PZQ ones. P5: between *U. tomentosa* mice & *U. tomentosa* + PZQ ones. P6: between PZQ mice & *U. tomentosa* + PZQ.

Table 4: Comparison between mean H- scores of NF-kB in groups

		N	$\bar{x}$ ± SD	ANOVA (F test)	Post Hoc test
H-Score of NF-kB in liver tissues	Infected	10	48.5±1.6	P<0.001**	P1: <0.001**
	U. tomentosa	10	22.3±1.8		P2: <0.001**
	PZQ	10	36.1±2.07		P3: <0.001**
	U. tomentosa + PZQ	10	21.1±1.79		P4: <0.001**
					P5: >0.05
					P6: <0.001**

Discussion

Egyptian medicinal plants have a marked role as anti-helminthes (Abo-Madyan *et al,* 2004), antiprotozoa (Rizk *et al.* (2019) as well as larvicidal action (Abdel-Hady *et al,* 2014) In the present study, PZQ-treated groups had the best significant reductions in worm load, tissue egg count, and number of liver granulomas.

U. tomentosa treatment group showed the lowest reduction percentage with relatively limited effectiveness. This agreed with authors confirmed the superiority of PZQ as anti-schistosomal drug. El-Lakkany *et al.* (2012) recorded that silymarin a product of artichoke lost to PZQ and the best results achieved when the extract was combined with PZQ. Also, Shaaban *et al.* (2019) reported that anti-schistosomal effect of PZQ was more efficient than *Crocus sativus* aqueous extract alone. However, other herbs extract recorded equal results or overcame PZQ's antiparasitic effects as *Pomegranata granatum, Commophora molmol* extracts *Allium sativum*, *A. cepa*, *Schitozim, Artemisia absinthium, Tanacetum parthenium, A. sativum* clove, *Zingiber officinale, Lavendula dentata* L., *Thymus capitatus* L. and *T. bovei* (El-Sherbini *et al,* 2009; Mantawy *et al,* 2011; El-Hela *et al,* 2013; Allan *et al,* 2014; de Almeida *et al,* 2016). Meanwhile, the opposite was noted in granuloma diameter, *U. tomentosa* treated groups gave highest granuloma diameter reduction either alone or with PZQ. But the significant difference between *U. tomentosa* treated group and PZQ treated group confirmed the ability of this extract to reduce granuloma diameter and protect the liver from damage. To our knowledge, none published about the relation between *U. tomentosa* extract and the *S. mansoni* granuloma diameter, but others supported the hepatoprotective effects of this extract and reduction of liver damage caused by hepatotoxic substances as fipronil (Abdelrazek *et al,* 2017) and carbon tetrachloride (Al-khayyal *et al,* 2019), as well as hepatoprotective effect due to its high antioxidant efficacy that prevented the inducible nitric oxide synthase gene expression (Batiha *et al,* 2020).

In the present study, the lowest mean H-scores of NF-κB expression in liver tissues was seen in both *U. tomentosa* treated groups. Liu *et al.* (2014) reported that prevention of hepatic fibrosis is intimately related to the inhibition of NF-kB signaling. Thus,

U. tomentosa can prevent hepatic fibrosis caused by *S. mansoni* infection. Also, Wan *et al.* (2017) found that formation of hepatic granulomas and fibrosis were primarily linked to NF-κB activation. Elgawish *et al.* (2019) reported that *U. tomentosa* decreased liver tissue immunostaining and liver damage via the inhibition of NF-κB. Baska and Norbury (2022) reported that of NF-κB and reduce the oxidative stress *S. mansoni* induced NF-κB activation in hepatic stellate cells associated with liver fibrosis.

The current results recorded that highest mean serum level of IL-4 and IL-5 in *U. tomentosa* treated groups. Schistosomiasis host developed an early Th1-polarized response as determined by increasing of IL-2, IFN-γ, & TNF-α, following oviposition precipitated Th2 immune response and subsequently raises the levels of IL-4, IL-5, IL-10, & IL-13 (Stadecker *et al,* 2004). Th2 cells play a crucial host-protective role during infection as they are a major constituent of granulomas developed around the eggs which sequestering eggs away from the surrounding liver tissues (Fairfax *et al,* 2012). The IL-4 has a potential protective function in schistosomaiasis and hepatocyte damage was more markedly exacerbated with high mortality rates in the IL-4-deficient mice (Fallon *et al,* 2000). But, IL-5 is important for eosinophil differentiation released from bone marrow recruitment into the tissues, and/or its activation in *S. mansoni* infected mice (Sher *et al,* 1990). The major component of granuloma is eosinophils with some neutrophils, fibroblasts, plasma cells and macrophages, blocking the action of IL-5 decreased granuloma size (Mourra *et al,* 2006). Domingues *et al*. (2011b) reported that the extract induces Th2 polarization, with increased levels of IL-4 & IL-5.

In the present study, lowest mean serum level of NO was in *U. tomentosa* treated groups, but highest one was recorded in infected non treated mice as compared to the healthy control. This agreed with Al-Olayan *et al.* (2016) who reported that schistosomiasis caused a significant increase in the levels of NO compared with control. *U. tomentosa* down regulated the expression of inducible nitric oxide synthase that lowered nitric oxide production and blocks cytotoxic effect and hepatic inflammatory pathway (Sandoval-Chacón *et al,* 1998). Thus, the present study showed best levels improvement in the ALT & AST in *U. tomentosa* received mice. This agreed with Abdelrazek *et al.* (2017) they reported that the hepatotoxic substance fipronil improved ALT & AST in *U. tomentosa* treated mice. Moreover, Al-khayyal *et al.* (2019) who reported hepatic injury were improved by carbon tetrachloride.

Conclusion

Undoubtedly, zoonotic schistosomiasis is one of the worldwide zoonotic parasites affecting the human welfare, and consequently must be eradicated.

Uncaria tomentosa extract alone cannot treat schistosomiasis *mansoni* in mice. However, it markedly has hepatoprotective effects through the liver enzymes and the reduction of NF-κB.

References

Abd El-Aal, NF, Hamza, RS, Harb, O, 2017: Paeoniflorin targets apoptosis and ameliorates fibrosis in murine *schistosomiasis mansoni*: A novel insight. Experimental Parasitology 183: 23-32.

Abdel Hady, NM, El-Sherbibi, GT, Morsy, TA, 2008: Treatment of *Toxoplasma gondii* by two Egyptian herbs. J. Egypt. Soc. Parasitol. 38, 3:1024-5.

Abdel-Hady, NM, El-Hela, AA, Morsy, TA, 2014: Phenolic content of some selected Lamiaceous Egyptian medicinal plants: Antioxidant potential and ecological friend mosquito-larvicidal. J. Egypt. Soc. Parasitol. (JESP); 44, 1:21-4.

Abdelrazek, HMA, Zeidan DW, Eltamany D A, Ebaid, HM, 2017: *Uncaria tomentosa* (cat claw) counteracts chronic fipronil-induced endocrine disruption induced insulin resistance and hepatic damage in male albino rats. Egypt. Acad. J. Biol. Sci. 9, 2:77-85.

Abo-Madyan, AA, Morsy, TA, Motawea, SM, Morsy, ATA, 2004: Clinical trial of Mirazid® in treatment of human fascioliasis in Ezbet El-Bakly (Tamyia Center) Al-Fayoum Governor-

ate. J. Egypt. Soc. Parasitol. 34, 3:807-18.
Abouel-Nour, MF, El-Shewehy, DMM, Hamada, SF, Morsy, TA, 2015: The efficacy of three medicinal plants; garlic, ginger and mirazid and a chemical drug metronidazole against *Cryptosporidium parvum*: I- Immunological response. JESP 45, 3:559-70.
Al-khayyal, RA, Al-Mousa, FA, Alaiary, AM, Alrassan, LA, Gado, AM, 2019: Beneficial effect of *Uncaria tomentosa* against CCl4-induced hepatotoxicity: Experimental study. Life Sci. J. 16, 9:42-9.
Allan, LA, Kutima, HL, Muya, S, Ayonga, D, Yole, D, 2014: The efficacy of an herbal drug, *Schitozim* over Praziquantel in the management of *Schistosoma mansoni* infection in BALB/c mice. Biol. Agric. Health J. 4, 1:77-86.
Al-Olayan, EM, El-Khadragy, MF, Alajmi, RA, Othman, MS, Bauomy, AA, *et al,* 2016: *Ceratonia siliqua* pod extract ameliorates *Schistosoma mansoni*-induced liver fibrosis and oxidative stress. BMC Complement. Altern. Med. 16: 434-8.
Ashour, DS, Shohieb, ZS, Sarhan, NI, 2015: Upregulation of toll-like receptor 2 and nuclear factor-kappa B expression in experimental colonic schistosomiasis. J. Adv. Res. 6, 6:877-84.
Baska, P, Norbury, LJ, 2022: The role of nuclear factor kappa B (NF-κB) in the immune response against parasites. Pathogens 11:310-6.
Batiha, GE, Beshbishy, AM, Wasef, L, Elewa, YHA, Abd El-Hack, ME *et al,* 2020: *Uncaria tomentosa* (Willd. ex Schult.) DC.: A review on chemical constituents and biological activities. Appl. Sci. 10:266-8.
de Almeida, LMS, de Carvalho, LSA, Gazolla, MC, Pinto, PLS, da Silva, MP, *et al,* 2016: Flavonoids and sesquiterpene lactones from *Artemisia absinthium* and *Tanacetum parthenium* against *Schistosoma mansoni* worms. Evid-Bas. Complement. Altern. Med. Article ID 9521349, 9 pages.
Domingues, A, Sartori, A, Golimc, MA, Valente, LMM, da Rosa, LC, *et al,* 2011a: Prevention of experimental diabetes by *Uncaria tomentosa* extract: Th2 polarization, regulatory T cell preservation or both? J. Ethnopharmacol. 137: 635-42.
Domingues, A, Sartori, A, Valente, LMM, Golim, MA, Siani, AC, *et al,* 2011b: *Uncaria tomentosa* aqueous-ethanol extract triggers an immunomodulation toward a Th2 cytokine profile. Phytother. Res. 25:1229-35.
Duvall, RH, DeWitt, WB, 1967: An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am. Trop. Med. Hyg. J. 16:483-6.
Elgawish, RA, Abdelrazek, HMA, Ismail, SA A, Loutfy, NM, Soliman, MTA, 2019: Hepatoprotective activity of *Uncaria tomentosa* extract against sub-chronic exposure to fipronil in male rats. Environ. Sci. Pollut. Res. 26:199-207.
El-Hela, AA, Abdel-Hady, NM, Dawoud, GT M, Hamed, AM, Morsy, TA, 2013: Phenolic content, antioxidant potential and *Aedes aegyptii* ecological friend larvicidal activity of some selected Egyptian Plants. J. Egypt. Soc. Parasitol. 43, 1:215-34
El-Lakkany, NM, Hammam, OA, El-Maadawy, WH, Badawy, AA, Ain-Shoka, AA, *et al,* 2012: Anti-inflammatory/anti-fibrotic effects of the hepatoprotective silymarin and the schistosomicide praziquantel against *Schistosoma mansoni* induced liver fibrosis. Parasit. Vectors 5:9-14.
El-Sherbini, GTM, El Gozamy, BR, Abdel-Hady, NM, Morsy, TA, 2009: Efficacy of two plants extracts against vaginal trichomoniasis. J. Egypt. Soc. Parasitol. 39, 1:47-58.
Fairfax, K, Nascimento M, Huang, SCC, Everts, B, Pearce, EJ, 2012: Th2 responses in schistosomiasis. Semin. Immunopathol. 34:863-71.
Fallon, PG, Richardson, EJ, McKenze, GJ, McKenzie, AND, 2000: Schistosome infection of transgenic mice defines distinct and contrasting pathogenic roles for IL-4 & IL-13: IL-13 is a profibrotic agent. J. Immunol. 164: 2585-91
Fraser, JA, Reeves, JR, Stanton, PD, Black, D M, Going, JJ, *et al,* 2003: A role for BRCA1 in sporadic breast cancer. Br. Canc. J. 88, 8:1263-70.
Fukushige, M, Chase-Topping, M, Woolhouse, MEJ, Mutapi, F, 2021: Efficacy of praziquantel has been maintained over four decades (from1977 to 2018): A systematic review and meta-analysis of factors influence its efficacy. PLoS Negl. Trop. Dis. 15, 3:e0009189
Ghazy, RM, Tahoun, MM, Abdo, SM, El-Badry, AA, Hamdy, NA, 2021: Evaluation of praziquantel efectivenss after decades of prolonged use in an endemic area in Egypt. Acta Parasitol. 66:81-90.
Global Burden of Disease Study (GBD Collaborators) 2017: Global, regional, and national incidence, prevalence, and years lived with disability for 328 diseases and injuries for 195 coun-

tries, 1990-2016: A systematic analysis for the Global Burden of Disease (GBD) Study 2016. Lancet 390, 1211-59.

Hailegebrie, T, Nibret, E, Munshea, A, 2021: Efficacy of praziquantel for the treatment of human schistosomiasis in Ethiopia: A systematic review and meta-analysis. J. Trop. Med. Article ID 2625255.

Herbert, DR, Orekov, T, Roloson, A, Ilies, M, Perkins, C, *et al,* 2010: Arginase I suppress IL-12/IL-23p40-driven intestinal inflammation during acute schistosomiasis. Immunol. J. 184, 11: 6438-46.

Hotez, PJ, Fenwick, A, 2009: Schistosomiasis in Africa: An emerging tragedy in our new global health decade. PLoS Negl. Trop. Dis. 3, 9: e485.

Keitel, WA, Potter, GE, Diemert, D, Bethony, J, El Sahly, HM, *et al,* 2019: A phase 1 study of the safety, reactogenicity, and immunogenicity of a *Schistosoma mansoni* vaccine with or without glucopyranosyl lipid A aqueous formulation (GLA-AF) in healthy adults from a non-endemic area. Vaccine 37:6500-9.

Lawrence, T, Fong, C, 2010: The resolution of inflammation: anti-inflammatory roles for NF-kB. Int. J. Biochem. Cell Biol. 42:519-23.

Liu, M, Wu, Q, Chen, P, Büchele, B, Bian, M, *et al,* 2014: A boswellic acid- containing extract ame-liorates schistosomiasis liver granuloma and fibrosis through regulating NF-κB signaling in mice. PLoS One 9, 6:e100129.

Mantawy, MM, Ali, HF, Rizk, MZ, 2011: Therapeutic effects of *Allium sativum* and *Allium cepa* in *Schistosoma mansoni* experimental infection. Rev. Inst. Med. Trop. Sao Paulo 53, 3: 155-63.

Manzella, A, Ohtomo, K, Monzawa, S, Lim, J H, 2008: Schistosomiasis of the liver. Abdom. Imag. 33:144-50.

Mourra, N, Leusurtel, M, Paye, F, Flejou, JF, 2006: Chronic schistosomiasis: An incidental finding in *Sigmoid volvulas*. J. Clin. Pathol. 59, 1: 111-4.

Peters, PA, Warren, KS, 1969: A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni*: Subcutaneous injection. Parasitol. 55, 3:55-8.

Reitman, S, Frankel, S, 1957: A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. Clin. Pathol. J. 28, 1:56-63.

Rizk, MA, El-Sayed, SAE, El-Khodery, S, Yokoyama, N, Igarashi, 2019: Discovering the in vitro potent inhibitors against *Babesia* and *Theileria* parasites by repurposing the Malaria Box: A review. Vet. Parasitol. 274:108895. doi: 10. 1016/j.

Sandoval-Chacón, M, Thompson, JH, Zhang, XJ, Liu X, Mannick, EE, *et al,* 1998: Anti-inflammatory actions of cat's claw: The role of NF kappa B. Aliment. Pharmacol. Ther. 12, 12:1279-89

Santos, KF, Gutierres, JM, Pillat, MM, Rissi, VB, dos Santos Araújo, MDC, *et al,* 2016: *Uncaria tomentosa* extract alters the catabolism of adenine nucleotides and expression of ecto 50-nucleotidase/ CD73 and P2X7 and A1 receptors in the MDA-MB-231 cell line. J. Ethnopharmacol. 194:108-16.

Shaaban, AM, Ibrahim, HM, Mohamed, AH, 2019: Effect of *Crocus sativus* aqueous extract (saffron) on *Schistosoma mansoni* worms in experimentally infected mice. Egypt. J. Aquat. Biol. Fisher. 23, 4:391-8.

Sher, A, Coffman, Rl, Hieny, S, Scott P, Cheever, AW, 1990: Interleukin 5 is required for the blood and tissue eosinophilia but not granuloma formation induced by infection with *Schistosoma mansoni*. Proc. Natl. Acad. Sci. USA 87: 61-5.

Silva-Moraes, V, Shollenberger, LM, Siqueira, LMV, CastroBorges, W, Harn, DA, *et al,* 2019: Diagnosis of Schistosoma mansoni infections: What are the choices in Brazilian low-endemic areas? Mem. Inst. Oswaldo Cruz, 114: e180478.

Stadecker, MJ, Asahi, H, Finger, E, Hernandez, HJ, Rutitzky, LI, *et al,* 2004: The immunobiology of Th1 polarization in high-pathology schistosomiasis. Immunol. Rev. 201:168-79.

Stephenson, R, You, H, McManus, D, Toth, I, 2014: Schistosome vaccine adjuvants in preclinical and clinical research. Vaccines 2, 3:654-85.

Wan, C, Jin, F, Du, Y, Yang, K, Yao, L, *et al,* 2017: Genistein improves schistosomiasis liver granuloma and fibrosis via dampening NF-kB signaling in mice. Parasitol. Res. 116, 4:1165-74.

WHO, 2018: Methods and Data Sources for Country-Level Causes of Death 2000- 2016. Global Health Estimates Technical Paper WHO/HIS/ IER/GHE/2018.3.file:///C:/Users/relborm/Desktop/Global COD; Geneva, Switzerland.

WHO, 2020: Schistosomiasis https://www.who.int/en/news-room/factsheets/detail/schistosomiasis. Geneva, Switzerland.

Explanation of figures

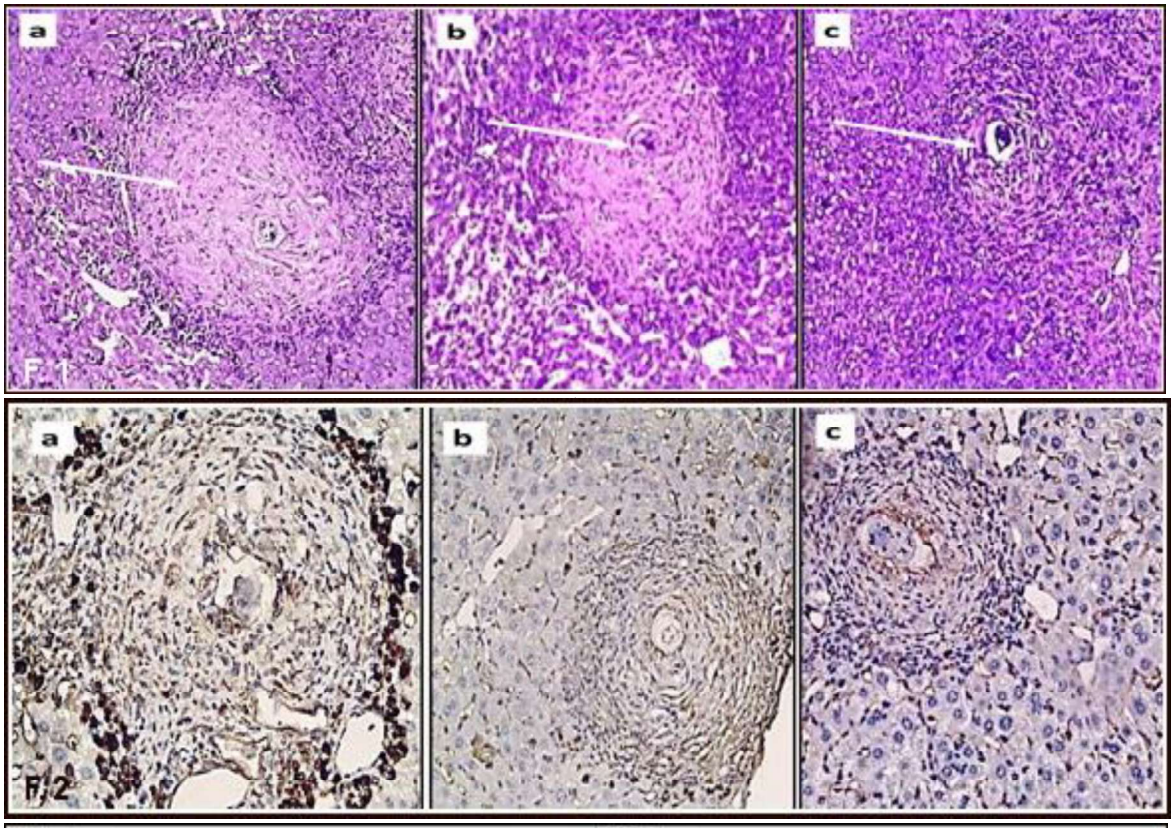
Fig. 1: H & E-stained liver tissue showed: a. Liver tissue of infected mice with large sized bilharzial granuloma (white arrow) with central ova surrounded by histiocytic and lymphocytic infiltrate. b. Liver tissue of PZQ treated mice with medium sized bilharzial granuloma (white arrow) with central ova surrounded by epithelioid cells surrounded by rim of lymphocytes. c. Liver tissue of *U. tomentosa* treated mice with small sized Bilharzial granuloma (white arrow) with central ova surrounded by histiocytic and lymphocytic infiltrate.

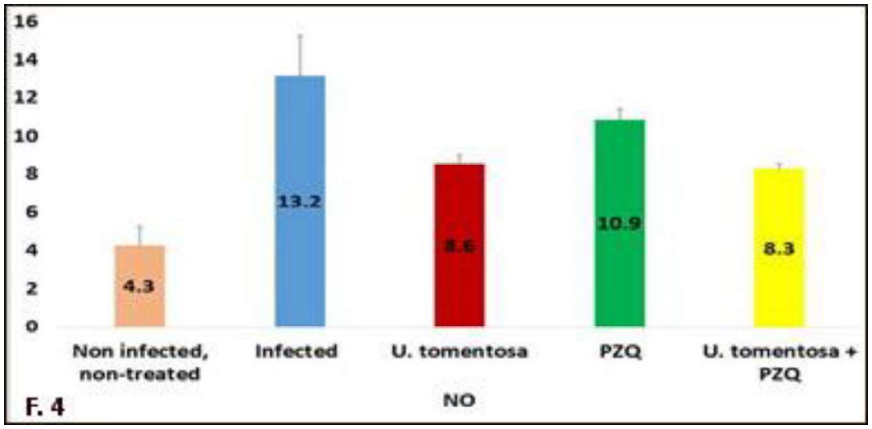
Fig. 2: a. NF-κB Immunostaining of liver tissue: a. Strong positive expression of NFKB in large sized granuloma of infected mice. b. Moderate positive expression of NFKB in medium sized granuloma of PZQ treated mice. c. low positive expression of NFKB in small sized granuloma of U. tomentosa treated mice.

Fig. 3: Comparison between groups regarding serum levels of IL-4 (A) and IL-5 (B)

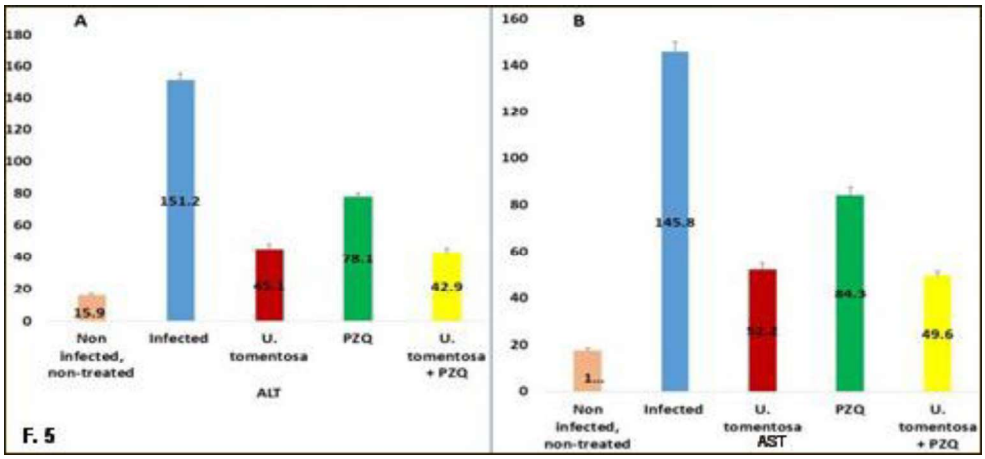
Fig. 4: Comparison between groups regarding serum levels of Nitric oxide.

Fig. 5: Comparison between groups regarding serum levels of liver enzymes ALT (A) and AST (B).




16
14
12
10
8
6
4
2
0
4.3
13.2
8.6
10.9
8.3
Non infected, non-treated
Infected
U. tomentosa
PZQ
U. tomentosa + PZQ
NO

F. 4


A
180
160
140
120
100
80
60
40
20
0
15.9
151.2
78.1
42.9
Non infected, non-treated
Infected
U. tomentosa
PZQ
U. tomentosa + PZQ
ALT
B
160
140
120
100
80
60
40
20
0
145.8
49.6
Non infected, non-treated
Infected
U. tomentosa
PZQ
U. tomentosa + PZQ
AST

F. 5