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EVALUATION THE ANTISCHISTOSOMAL AND ANTIOXIDANT POTENTIAL OF C. PAPAYA FRUIT EXTRACTS AGAINST SCHISTOSOMA MANSONI.

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ABSTRACT

Schistosomiasis is a major disease of public health in human. Patterns on immune response, worm recovery, in vitro antischistosomal bioassay screening (cercaricidal killing in vitro of Schistosoma mansoni was observed). In vivo Mice were infected by injection of 100 S. mansoni cercariae/mouse and treated by oral administration with crude extract, MeOH, ethyl acetate (EtOAc) and butanol (BuOH) Carica papaya fruits (4g/kg) start from 7th day p.i. to the end of experiment and a treatment control of 500 mg/kg of Praziquantel. Various concentrations of plant extracts were used in cercaricidal assay. Both crude extract and EtOAc showed significant dose-dependent percentage worm load reduction (P<0.001). Carica papaya, showed highly reduction in worm counts (60.3% and 68.2%) while Praziquantel (92.8%), elevated immune responses and least time in destroying cercariae. The highest significant reduction in dead ova (P<0.001) was observed in group treated with EtOAc extract. The level of IgG1 and IgG2 was significantly reduced (P<0.001) than in groups treated with Carica papaya crude extract, EtOAc extract or PZQ as compared to levels in untreated infected mice. While the level of IgG1 and IgG2 was insignificantly reduced in groups treated with MeOH extract or BuOH extract as compared to levels in untreated infected mice.

Conclusion: *The antischistosomal Efficacy of the extracts was dependent being more potent in reducing both the worm burden and tissue egg load. The antishistosomal effect of Carica papaya extracts was significantly higher in group treated with EtOAc extract. These findings successive the potential use of Carica papaya extracts in the management of schistosomiasis and provide a basis for exploring medicinal plants as sources for new antischistosomal agents.*

Keywords: Schistosomiasis- Carica Papaya-Praziquantel-Antioxidant Potential.

Introduction:

Carica papaya L. (papaya) is member of family Caricaceae and widely cultivated for its edible fruits **(1).** *C. papaya* is one of the most important fruit crops grown in the tropical and sub-tropical regions worldwide **(2).** *C. papaya* fruit is the major product from the tree and it is well known for its excellent taste, nutritive value and its digestive effects **(3).** In the market, there is an increase interest of products derived from papaya in food and drug industry **(4).** Many scientific investigations showed that, *C. papaya* fruits extracts have many health benefits, such as reducing cardiovascular disease risk, anti-inflammatory, antioxidant, anticancer, antimicrobial activities and serving an immune-adjuvant for vaccine therapy **(5, 6, , &7).** *C. papaya* phytochemical studies showed the presence of biologically and pharmacologically active constituents such as carotenoids, phenolic acids, flavonoids, and vitamin C **(8).** Considering the vast potentiality of plants as sources for anthelminthic drugs with reference to antiscistosomal agents, a systematic investigation was undertaken to screen the antischistosomal activities (in vivo) from dried seeds of *Carica papaya*. Their methanol and aqueous extracts were evaluated for antischistosomal properties against Schistosoma mansoni (**9**). *Carica papaya* as potential natural medicinal source. Several studies on methods used in extracting *Carica papaya* materials from different parts of the plant were highlighted. Extracts from different parts of *Carica papaya* plant have shown protective effects against many diseases such as intestinal worms infection and different types of wounds. Extracts also showed positive effects when used as antiparasitic, antiseptic, antimicrobial, anti-inflammatory, antihyperlipidemic, antihypertensive and antidiabetic **(10)**. The in vivo antiprotozoal activity of crude C. papaya seeds extract and its main components against T. cruzi infective forms (blood trypomastigotes and amastigotes), during the acute phase of the disease was evaluated by **(11).** Praziquantel still not reaching the majority of those who most need it due to its high cost and there is possibility of drug resistance, hence need for alternatives (**12**). Praziquantel (PZQ) remains the only antibilharzial drug effective against the four main schistosomes pathogenic to man (**13**&**14**). Although it has been reported that PZQ has minimal side effects (**15 and 16**). Control of schistosomiasis using PZQ at a population level faces some problems. Resistance to PZQ has been recently induced in schistosomes by laboratory selection (**17**). Reduced cure rates and failure of treatment after PZQ have been reported in Senegalese, Kenyan and Egyptian patients Praziquantel still not reaching the majority of those who most need it due to its high cost and there is possibility of drug resistance, hence need for alternatives. The main aim of this study was to carry out a phytochemical analysis of *C. papaya* fruit methanolic extract using HPLC-ESI-MS

technique. Evaluation the antischistosomal properties of *C. papaya* fruit extracts against *Schistosoma mansoni* as well as their antioxidant potential.

Material and methods

Plant material

Carica papaya fruits were purchased from local market, Giza, Egypt in May 2015. The voucher plant sample was characterized by Prof. Dr. Wafaa Amer, Professor of plant taxonomy, Faculty of Science, Cairo University. The voucher specimen has been deposited in medicinal chemistry laboratory, Theodor Bilharz Research Institute. The fruits of *C. papaya* were cut to small pieces, dried in the shade, finely powdered with an electric mill, and the dry powder was kept for the extraction process.

Extraction and fractionation process

Finely powdered *C. papaya* fruits (700 g) were extracted with 4 liters of 85 % MeOH at room temperature. 85% MeOH extract was filtrated and concentrated to dryness under reduced pressure using a rotatory evaporator (BUCHI, Switzerland) for three times. 85 % MeOH extract was defatted with petroleum ether, and the aqueous defatted MeOH extract was subjected to fractionation using dichloromethane (CH_2Cl_2), ethyl acetate (EtOAc) and butanol (BuOH) respectively. The three fractions were concentrated to dryness with a rotatory evaporator. The methanolic extract and the three fractions were kept away from any moisture.

In vitro study

1. *In vitro* antischistosomal bioassay screening

Schistosoma mansoni worms were purchased from the Schistosoma Biological Supply Center (SBSC) at Theodor Bilharz Research Institute (TBRI), Giza, Egypt. The antischistosomal assay was carried out using method described by **(18)**. *S. mansoni* worms were washed several times in sterile RPMI-1640 media (Cutilab) (pH 7.5, with HEPES 20 mM and supplemented with penicillin (100 U/mL), sterptomycin (100 mg/mL), and 10% fetal calf serum **.** In 35 mm diameter (35 × 10 mm) polystyrene petri dish, 10 adult *S. mansoni* worms were cultured in 10 mL sterile RPMI-1640 media with descending concentrations of plant extracts and oil(500, 250 and 125 μg/mL) then incubated in a humid 5 % CO_2 shaking incubator **(**SSI10R Large Refrigerated Incubator Shaker, Germany**)** at 37 °C for 24 hrs. In parallel, the adult worms were cultured in RPMI-1640 media containing 10% DMSO (Served as solvent control). The efficacy of different concentrations of plant extracts, mortality, viability and shrinking of worms, was observed using a stereomicroscope at different time intervals including 1 h, 3 and 24hrs of incubation.

2. Animals

Six to eight week old male albino mice of the CD1 mice (weight 24 ± 2g) bred and kept at the Schistosome biological supply center, Theodore Bilharz Research Institute Giza, Egypt (SBSP/TBRI). The mice were bred under environmentally controlled conditions, fed with a standard pellet diet and distilled water. Handling and treatment of animals were conducted according to internationally valid guidelines and ethical conditions adopted by Theodore Bilharz Research Institute.

Doses

Crude or purified extracts were administered in doses of 4g/Kg body weight daily for 45 days in a standard pelleted diet containing 24% protein, 4% fat and about 4-5% fiber according to. Praziquantel was administered in a dose of 500 mg/kg body weight on two successive days after 45 days of infection .

3. Parasites

S. mansoni cercariae were obtained from Schistosome biological supply center, TBRI, and infection was performed directly after shedding from ***Biomophlaria alexandrina snails.***

4. Experimental Groups

A batch of 70 mice was divided into six groups as follow:

Group 1: Normal healthy control (10 mice).

Group 2: Infected control group (10 mice). Mice were infected by the subcutaneous (s.c.) injection of 100 ***S. mansoni*** cercariae/mouse.

Group 3: Infected treated group (10 mice). Mice were infected by (s.c.) injection of 100 ***S. mansoni*** cercariae/mouse and treated by oral administration with crude *Carica papaya* fruits (4g/kg) start from 7th day p.i. to the end of experiment.

Group 4: Infected treated group (10 mice). Mice were infected by (s.c.) injection of 100 ***S. mansoni*** cercariae/mouse and treated by oral administration with MeOH extract (4g/kg) start from 7th day p.i. to the end of experiment.

Group 5: Infected treated group (10 mice). Mice were infected by (s.c.) injection of 100 ***S. mansoni*** cercariae/mouse and treated with EtOAc extract (4g/kg) start from 7th day p.i. to the end of experiment.

Group 6: Infected treated group (10 mice). Mice were infected by (s.c.) injection of 100 ***S. mansoni*** cercariae/mouse and treated with BuOH extract(4g/kg) start from 7th day p.i. to the end of experiment

Group 7: Infected treated group (10 mice). Mice were infected by (s.c.) injection of 100 ***S. mansoni*** cercariae/mouse and treated twice at 6 weeks post-infection (p.i.) with 500 mg/kg PZQ.

All mice were sacrificed at 8 weeks post-infection and subjected to the following parameters.

Parasitological Criteria

1. Worm burden:

Adult worms were harvested by hepatic and intestinal

perfusion 6, 8, and 16 weeks after infection according to the method described by **(19).**

2. Tissue egg load (liver and intestine)

The number of eggs per gram tissue (liver and intestine) was studied according to the procedure by **(20).**

3. Percentage egg developmental stages "Oogram Pattern":

The percentages of immature, mature, and dead ova in the small intestines were computed from a total of 100 eggs per intestinal segment and classified according to the categories previously defined by **(21).**

Immunological Parameters:

Determination of anti-Schistosomal immunoglobulin subclasses IgG1, IgG2 and IgG4 were measured using indirect ELISA, based on the method of **(22)**. ELISA microtiter plates were coated with 100 ul / well of 30 ug/ml of soluble worm antigen. Sera were diluted 1:20 and anti-mouse IgG subclasses (Binding site, Birmingham, UK) were used at a dilution of 1:500. Absorbance at 492 nm was measured.

Statistical analysis: The data were presented as mean standard error of the mean (X±SE).

The means of the different groups were compared globally using the analysis of variance

ANOVA. Data were considered significant if p values were less than 0.05.

Results

Worm load:

The worm burden and tissue egg load in the intestine and liver were calculated for each studied group (Table 1). In the infected control group, the total number of worms counted was 29.2 ± 0.99. Oral administration of crude extract of *Carica papaya* , MeOH extract, EtOAc extract or BuOH extract of *Carica papaya* (100 mg/kg) to mice after infection reduced the total worm burden to 11.6 ± 1.44 (60.3 % reduction), 17.1 ± 0.29 (38.7%reduction) and 9.3 ± 1.21 (68.2% reduction) and 13.8 ± 2.09 (52.7 % reduction) respectively whereas, administration with 500 mg/kg PZQ on two consecutive days at six weeks post-infection reduced the total worm burden to 2.1 ± 0.03 (92.8% reduction).Oral administration of crude extract of *Carica papaya* , MeOH extract, EtOAc extract or BuOH extract of *Carica papaya* (100 mg/kg) to mice after infection reduced egg load both in the intestine and liver to (51.7 % & 50.9 % reduction), (33.7% & 33.5% reduction), (40.2% & 71.9% reduction) and (43.9 % & 66.5 % reduction) respectively.Whereas, administration with 500 mg/kg PZQ on two consecutive days at six weeks post-infection reduced the egg load both in the intestine and liver to (96 % & 95.6 % reduction). (Table 1) (fig.1).

Table 1: Worm burden and tissue egg load in mice treated with crude andsoluble fractions of *C. papaya*.

Animal group	Mean no. of worms ± SEM	% reduction	Mean no. of ova count ± SEM / g tissue			
			Intestine	% reduction	Liver	% reduction
Infected control	29.2 ± 0.99		17839 ± 1981		6519 ± 704	
Treated group						
Crude extract	11.6 ± 1.44	60.3%	8619 ± 168	51.7%	3198 ± 29	**50.9%**
MeOH extract	17.9 ± 1.91	38.7%	11829 ± 443	33.7%	4332 ± 37	**33.5%**
EtOAc extract	9.3± 1.21	68.2%	7104 ± 259	40.2%	1829 ± 23	**71.9%**
BuOH extract	13.8 ± 2.09	52.7%	9998 ± 274	43.9%	2183 ± 31	**66.5%**
PZQ	**2.1 ± 0.03**	**92.8%**	**793 ± 99**	**96 %**	**287 ± 9**	**95.6%**

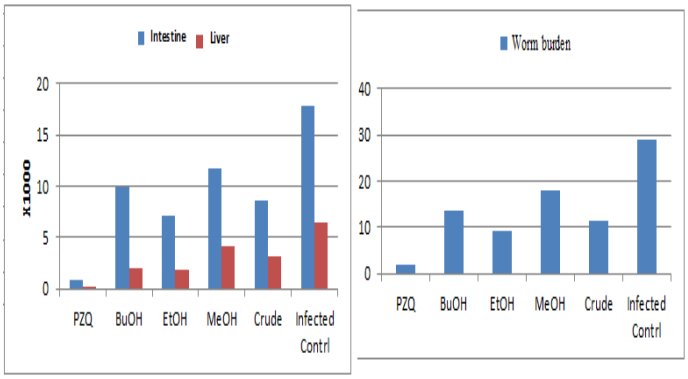

Fig.(1) Worm burden and tissue egg load in mice treated with crude and soluble fractions of *C. papaya*.

The results obtained in the current study showed a highly significant reduction (P<0.001) in the mean number of worms in infected and treated with EtOAc extract group IV and crude extract of *Carica papaya*II compared to the infected untreated group. Also the current study showed a moderate significant reduction (P<0.05) in the mean number of worms in group treated with MeOH extract and in group treated with BuOH extract compared to the infected untreated group.

Oogram pattern:

The percent of immature ova was in significantly difference in all treated groups than the infected untreated one. While the percent of dead ova was (11.6 %, 17.2, 27.1% & 10.7) in the groups treated with crude extract of *Carica papaya*, Me OH extract, EtOAc extract or BuOH extract of *Carica papaya* respectively. The highest significant reduction in dead ova (P<0.001) was observed in group treated with EtOAc extract. (Table.2).

Table 2:- Egg developmental stages (oogram) of infected mice administrated with crude and soluble fractions of *C. papaya*.

Animal Group	Oogram pattern (% ova)		
	Immature	Mature	Dead
Infected control	55.8 ± 3.8	39.1 ± 2.6	**5.1 ± 0.3**
Crude extract	59.6 ± 2.9	28.8 ± 3.7	**11.6 ± 0.09**
MeOH extract	49.9 ± 3.1	32.9 ± 2.9	**17.2 ±0.1**
EtOAc extract	52.1 ± 4.2	20.8 ± 2.3	**27.1 ± 0.32**
BuOH extract	61.5 ± 3.3	.8 ± 1.127	**10.7 ± 1.1**
PZQ	**22.0 ± 0.3**	**8.9 ± 0.2**	**69.1 ±4.9**

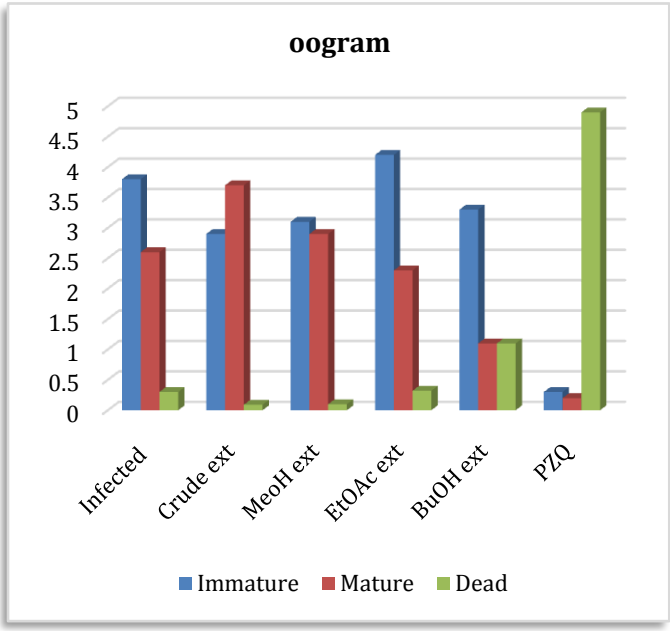

Fig. (2) Egg developmental stages (oogram) of infected mice administrated with crude and soluble fractions of *C. papaya*

Immunological Parameters:

Fig. (3) Showed the level of sera immunoglobulin subclasses IgG1, IgG2 and IgG4 in samples of mice infected with *S. mansoni.* The level of IgG1 and IgG2 was significantly reduced (P<0.001) than in groups treated with *Carica papaya* crude extract, EtOAc extract or PZQ as compared to levels in untreated infected mice. While the level of IgG1 and IgG2 was insignificantly reduced in groups treated with MeOH extract or BuOH extract as compared to levels in untreated infected mice. In regard to the level of IgG4 there is no any significant difference between all treated groups as compared to levels in untreated infected mice.

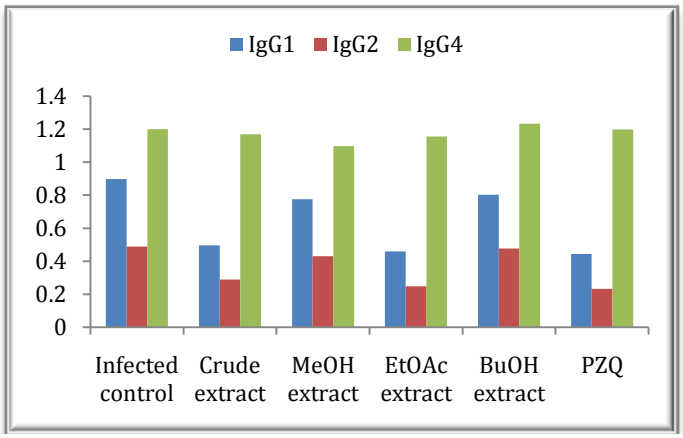

Fig. (3) level of sera immunoglobulin subclasses IgG1, IgG2 and IgG4 in samples of mice infected with *S. mansoni* administrated with crude and soluble fractions of *C. papaya*..

DISCUSSION:

Control of helminthiasis has therefore been the center of focus in biomedical research since time immemorial. Both the medical and veterinary professions have tried to control helminthiasis by administration of synthetic drugs **(23)**. The anthelmintic property of plants is dependent on numerous substances that are found in them. These could be alkaloids, sugars, saponins, aromatic oils, resins and other medicinally useful chemicals (**24**). The milky juice of *Carica papaya* contains proteolytic ferments, which together with papain have successfully been used as an anthelmintic agent for the treatment of Ascariasis, Trichuriasis, and ancylostomiasis (**25**).

This study agree with **(9)** they showed that Significant effect of the extracts was observed against schistosomal infected mice. *Carica papaya* methanol extract was found more effective against schistosomes recording less recovery while *Carica papaya* aqueous extract recorded more recovery.The results of worm maturation in this study are comparable to those of other studies using the same mouse model confirming swiss mice as a good model for schistosome studies. The greatest loss of larval stages occurs during the migration through the lungs with relatively smaller losses during migration through the skin **(26)**

The antischistosomal properties of *C. papaya* fruit extracts against *Schistosoma mansoni* as well as in vitro antischistosomal bioassay screening their antioxidant potential were evaluated.

Antischistosomal effects of crude *Carica papaya*(methanol or aqueous) extracts were studied Patterns on immune response, worm recovery, gross pathology in vivo and cercaricidal killing in vitro of *Schistosoma mansoni* was observed.

In the present study, *C. papaya* methanol extract exhibit the shortest time to kill cercariae compared to *C. papaya* aqueous extract, this is agree with **(12)**

The maximum duration for the destruction of the cercariae

in the four treatments; both aqueous and methanol treatments of *C. papaya* was 20 minutes in the lowest concentrations (5 µg/ml). Time of killing decreased with increase in concentrations to a maximum concentration (30 µg/ml). The speed, at which cercariae can penetrate skin and find a vascular portal, varies considerably. The maximum killing time (20 minutes) was very encouraging because it is less than the time taken by most cercariae to locate and penetrate the host skin **(27)**. A few cercariae can make this journey within five minutes **(28)** in which they would have already been weakened or killed by the extracts. The ability of these extracts to destroy cercariae can be incorporated in an ointment to be applied by people before wading in water infested with schistosome infected snails.

In the present study, the level of IgG1 and IgG2 was significantly reduced (P<0.001) in groups treated with *Carica papaya* crude extract, EtOAc extract or PZQ as compared to levels in untreated infected mice. While the level of IgG1 and IgG2 was insignificantly reduced in groups treated with MeOH extract or BuOH extract as compared to levels in untreated infected mice.*Carica papaya*, showed elevated immune responses and least time in destroying cercariae **(12)**.The elevated levels of IgG responses in infected-untreated control can be associated with a high worm burden leading to a high level of circulating parasite antigens many of which are not related to protection **(29)**. This high IgG level did not confer protective immunity in infected-untreated control as demonstrated by the highest number of worm recovery. The IgG responses in Praziquantel were relatively high, and in this case, unlike the untreated control, it had the lowest worm burden and the lowest pathology. Praziquantel kills the worms directly and also, induces schistosome-specific immune response which reduces the worm burden further. This results in reduced pathology, as lower number of worms translates to lower egg production, and hence fewer granulomas**(12)**.*Carica papaya* methanol had lower IgG responses to both antigens as compared to aqueous extract, and lower worm counts, but pathology of both *C. papaya* extracts was similar. This high IgG response level seen in *C. papaya* and reduced gross pathology is supported by **(30)** who reported that *Carica* seed extract has an immunostimulatory action which is illustrated in the ability to inhibit significantly the classical complement-mediated haemolytic pathway.

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