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RESEARCH ARTICLE

Evaluating the Antischistosomal Activity of Crude Extracts of *Carica Papaya* against *Schistosoma Mansoni*: the Interplay of Cellular and Humoral Immunity

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ABSTRACT

Carica papaya is widely used in different parts of Kenya for the treatment of intestinal helminthes. The present study was designed to evaluate the anti-schistosomal effect of a methanolic and aqueous extract of *Carica papaya* seeds in schistosoma infected mice. Laboratory mice were infected with a single dose of *Schistosoma mansoni* cercariae. The extracts were administered orally at a dose of 300 mg/kg body weight in 200µl suspension to infected mice two days apart. Praziquantel was the reference drug used in the experiments.Two weeks post-treatment; all animals were sacrificed to evaluate the efficacy of *Carica papaya* in treatment of the infection. Significant effect of the extracts was observed against schistosomal infected mice. *Carica papaya* methanol extract was found more effective against schistosomes recording more less recovery while *Carica papaya* aqueous extract recorded more recovery. Detectable levels of cytokines were also recorded during infection and after treatment with a marked rise in SWAP specific IL5. These data support the use of *Carica papaya* based medicines as antischistosomal agents where acces to commercial drugs is restricted. These findings provide solid scientific evidence to support the traditional medicinal uses of these extracts and indicate a promising potential of this plant for medicinal purposes. There is also need for detailed scientific study of traditional medicinal practices to ensure that valuable therapeutic knowledge of this plant is preserved and also to provide scientific evidence for their efficacy.

KEY WORDS: Plant extract, Antischstosomal activity, *Schistosoma mansoni*, *Carica papaya*

INTRODUCTION:

Schistosomiasis is a parasitic disease caused by several species of trematodes (platyhelminth infection, or "flukes"), a parasitic worm of the genus *Schistosoma*. Among human parasitic diseases, schistosomiasis ranks second behind malaria in terms of socio-economic and public health importance in tropical and subtropical areas[1]. The disease is endemic in many developing countries, infecting more than 207 million people, 85% of who live in Africa. They live in rural agricultural and peri-urban areas, and placing more than 700 million people at risk[2]. The disease affects many people, particularly children who may acquire the disease by swimming or playing in infected water. As children come into contact with the contaminated water source the parasitic larvae easily enter through the human skin and further mature within organ tissues[3].

The control of schistosomiasis requires large scale population based chemotherapy in addition to environmental and behavioural modification. Schistosomiasis is readily treated using a single oral dose of the drug praziquantel (PZQ) annually a critical part of community-based schistosomiasis control programs[4,5,6,7]. However, resistance to it may be emerging after nearly 20 years of intensive use [8,9,11].

The use of medicinal plants as a source for relief from illness is doubtless an art as old as mankind. Before the introduction of modern medicines, disease treatment was managed entirely by herbal remedies. There are many medicinal herbs which find place in day –to –day uses, many of which are used as herbal remedies. It is estimated that about 80 percent of the world population residing in the vast rural areas of the developing and underdeveloped countries still rely on medicinal plants[12]. This has been as a result of the development of resistance, cross-resistance and possible toxicity hazards associated with conventional drugs and their rising costs. Medicinal plants are the only affordable and accessible source of primary health care for them. Phytochemicals obtained from the huge diversity of plant species are important source for safe and biodegradable chemicals, which can be screened for antischistosomal activities tested for mammalian toxicity[9,10].

Studies on both the methanol and aqueous extracts processed from the dried roots of *Solanum incanum* have shown protection against the effects of *Schistosoma mansoni.* In particular, the extracts have

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shown significant reduction in worm recovery i.e it is a potential antischistosomal agent, it has also significant immunological effects[13]. The crushed seeds of the plant *Nigella sativa* have also been found to have antischistosomal activity against different stages (cercariae and juvenile) of *S. mansoni in vitro*[14]. Tests carried on vernodalin, a highly toxic sesiquiterpene lactose compound, extracted from *Vernonia amygdalina* also showed significant activity against schistosomes as well as Plasmodium and Leishmania species. Myrrh, a gum extract from the stem of *Commiphora myrrha* (molmol-Somali) of the family Burserceae has been used to treat Schistosomiasis[15]. An extract of *Commiphora molmol* (myrrh) has been licensed and marketed for clinical use against Fasciola and schistosome infections in Egypt. The extract has some antischistosomal properties that cause worm pairs to separate. The female worms then shifts to the liver, where they are destroyed[15].

Considering the vast potentiality of plants as sources for anihelminthic 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.* This is in pursuance of the efforts to search for drugs from plants and the verification of the scientific basis of some known practices in traditional medicine.

MATERIALS AND METHODS:

COLLECTION OF THE PLANT MATERIAL:

Mature and ripe pawpaw fruits were obtained from the Jomo Kenyatta University of Agriculture and Technology farm, the seeds collected, stored in plastic bags and transported to the laboratory for processing. Taxonomic identification of the plant was done by Botany department and a voucher specimen was deposited in the department. Seeds were dried at room temperature (25˚C) for one month. After complete drying, the seeds were pulverized into small particles using Mekon Micromiller Single phase and passed through a 0.5mm mesh to standardize the particles. Two kilograms of the ground plant material was separately placed in different clean large bottles.

PREPARATION OF THE CRUDE EXTRACT:

Grounded fine powder separately soaked in 98% methanol and aqueous for 72hr and 36hr respectively and stirred occasionally was filtered using Whartman No. 1 filter paper. The aqueous filtrate was lyophilized using a freeze drying machine for a month after which the extract was obtained in powder form. The methanol filtrate was processed using a rotary vacuum evaporator at 70°C, and methanol was further removed by placing the samples on a water bath until there was no evaporation (methanol extract).

HOSTS AND PARASITES:

Swiss white mice about 30g and 7 weeks old were used in this study. The animals were acquired from the Kenya Medical Research Institute (KEMRI). The animals had free access to a standard commercial diet and water ad libitum and were kept in cages (same sex), maintained under standard conditions (12:12h light/dark cycle; ambient temperature (20˚C); 50-60% relative humidity) and maintained with free access to standard mice pellet diet and water made available *ad libitum*. A Kenyan isolate of *Schistosoma mansoni* , originally derived from infected humans and maintained under laboratory conditions in *Biomphalaria pfeifferi* and olive baboon was used to infect mice.

PREPARATION OF PARASITE ANTIGENS:

O-3HR ANTIGEN:

Schistosoma mansoni cercariae were obtained by shedding infected snails with a patent infection period of five weeks. The heads and tails of the cercariae were separated as described by Ramolho-Pinto *et al* (1974)[16]. The supernatant containing the proteins released by penetrating schistosomules between 0–3hr of penetration was aliquoted in cryovials. The protein content was determined using the Bradford method[17]. The antigen was sterilized by exposure to UV light for 10 min, aliquoted and stored at –20^{o}C.

On the other hand, Schistosome soluble worm antigen (SWAP) was prepared from 6-week-old *S. mansoni* worms recovered from infected mice. The worms were washed twice in PBS, and sonicated (24 kHz, 16mm amplitude, 10 min). The suspension was then centrifuged at 1x10^{5} g for 1 h, at 4°C to obtain the soluble protein fraction. Determination of the protein content and subsequent storage was done as for 0-3hr antigen.

MICE INFECTION AND TREATMENT:

Mice were infected with *S. mansoni* (250 cercariae/mouse) percutaneously via abdominal skin using the ring method[18]. Thirty days post-infection, the mice were divided into four subgroups of 18. In two groups, mice were separately (n=6) and individually orally treated with 300 mg/kg body weight in 200µl suspension, of either aqueous or methanol extracts of *Carica papaya* two days

apart. The other two groups served as controls; one infected and not treated and the other infected and dosed by 900mg/kg of praziquantel two days apart[19]. Two weeks post-treatment, all animals were sacrificed to evaluate the efficacy of *Carica papaya* in management of *S. mansoni* of the infection.

SAMPLING PROCEDURES:

At week six following infection of mice with parasites, sera and lymphocytes were harvested from six mice in each group. The six mice from each group were euthanized, the thoracic cavity opened, blood obtained by cardiac puncture and serum prepared for the antibody enzyme linked immunorsorbent assay (ELISA). The mice were also perfused and worm recovery determined according to the method described by Smithers and Terry[17]. Inguinal and auxillary lymph nodes and spleens were obtained for cell preparation. Pathological changes in liver and *S. mansoni* infected groups were also observed.

PERFUSION AND WORM RECOVERY:

Based on the modified method of Smithers and Terry[18], mice were anaesthetised and hepatic portal vein incised. Perfusion needle containing perfusion fluid (0.85% Sodium chloride and 1.5% Sodium citrate) was inserted on the left ventricle of the heart and perfusion carried out until the liver, lower limbs and mesenteries were clear. The perfusate was collected in plastic container and transferred in a urine jar to settle. The supernatant was sucked out and worms recovered [20]. The mean and percentage worm reduction of adult worms recovered for each group was calculated. Worm maturation was also calculated for the control groups.

ANTIBODY ASSAY:

Nunc-Immuno™ plates (MaxiSorp ™ Surface) ELISA plates were coated overnight with 50 µl of either SWAP (20ug/ml) or 0-3hr (10ug/ml) release protein antigen diluted in bicarbonate buffer pH 7.2 and incubated overnight at 4°C. The antigens were then dispensed off on a blotting paper. Non-specific binding was blocked by incubation with 100µl of 3% BSA in PBS for 1hr at 37°C. Mice sera from each mouse per group were then serially diluted at a factor of 1:5. Negative control was set up using sera of naive animals and positive controls using sera from mice infected with *S. mansoni*. Each well contained 100µl of diluted serum. IgG binding was detected using 100µl of rabbit anti-mouse horseradish peroxidase conjugate (1/5000; Sigma, UK). All the incubations were for 1h with appropriate washing steps using the washing buffer (0.05 % Tween 20 in PBS).The substrate; (50ul TM microwell peroxidase) was added to each well at100µl/well. The plates were incubated at 37°C in the dark for 30min. Optical density was read at 630nm in an ELISA microplate reader.

PROLIFERATION ASSAY:

Spleens were sampled individually from each mouse, for all the four groups of mice. Spleen was removed from each euthanized mouse and transferred to a petri dish containing sterile incomplete RPMI 1640 medium (RPMI1640, 0.1% Gentamycin, $5x10^{-5}$Beta mercap toet han ol). Using a 10ml syringe piston, the spleen was squashed through a fine wire mesh. Each cell suspension was dispersed with a sterile Pasteur pipette, sucked and dispensed into a 15ml sterile test tube. The cells were washed twice by centrifugation at 450 g for 10 minutes at room temperature and lymphocyte viability was determined by the trypan blue exclusion test and enumeration was done using a haemocytometer. Their concentration was made up to $3x10^6$ cells in 1 ml of complete RPMI 1640 medium (incomplete medium fortified with 10% fetal calf serum).

Lymph node cells were prepared by teasing the organ using sterile forceps in petri dish containing sterile incomplete medium. The cells were prepared for culture based on the procedure used for spleen cells.

Spleen cells and lymph node cells were cultured in flat-bottomed 48-well microtitres plates (Nunclon, Denmark). Duplicate wells were set for each regime, at a density of 3 x 10^5 viable cells. Negative wells contained medium and cells only, while positive control wells 1 µg Concanavalin A (Con A). The test wells of each plate contained 1µg/well of soluble worm antigen preparation (SWAP) or 0.5 pg/well of 0-3hr soluble antigen preparation (0-Hr). The total volume of culture medium per well was made up to 200ul. After culturing the cells for 48h (Con A) or 72 h (antigens) at 37°C, in a humidified incubator supplied with 5% CO2, 18.5 kBq [^{3}H] thymidine (specific activity 185 GBq; Amersham) was added to each well. After 18h, cells were harvested and the incorporated label measured by liquid scintillation counting. IFN-γ and IL-5 levels were determined by antibody-capture ELISA (MABTECH AB, Sweden). The optical density was measured at 630 nm using a Bio-Rad ELISA plate reader. Each plate included a standard curve based on serial dilutions of recombinant standard IFN-γ or IL-5 as required.

STATISTICAL ANALYSIS:

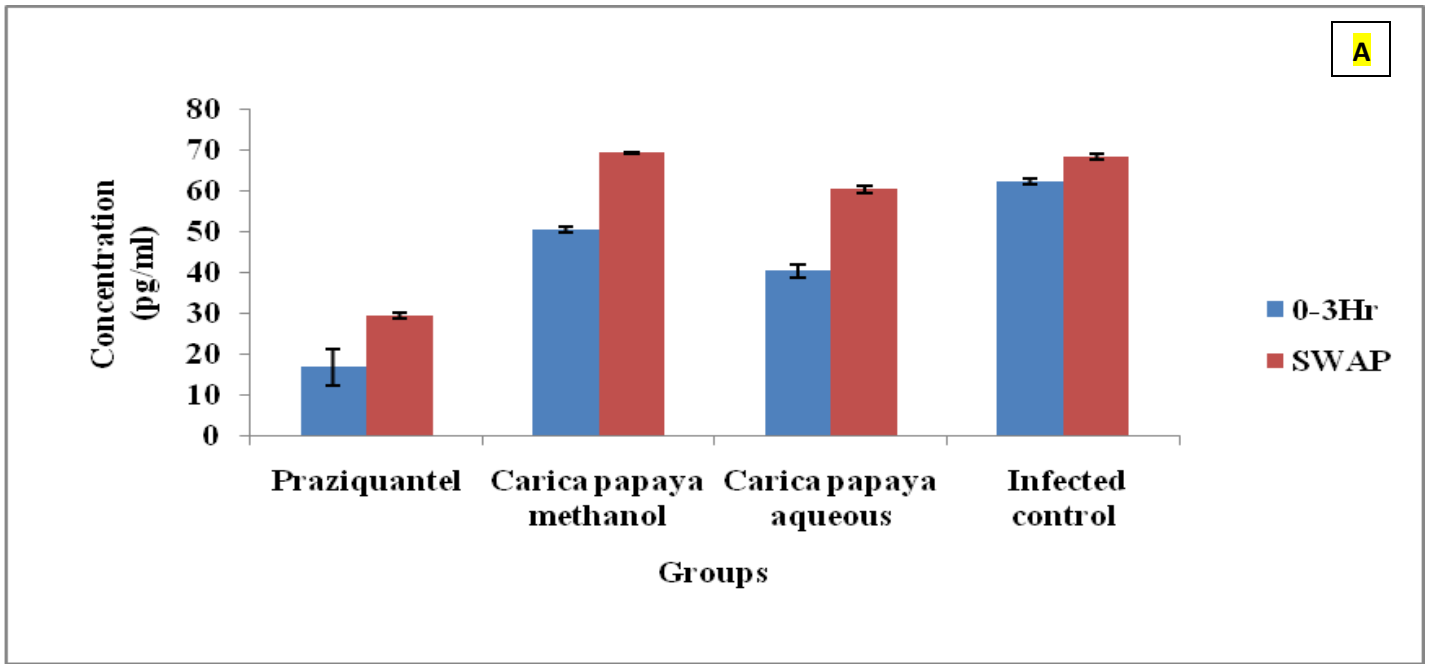
Student's t-test was used to compare means between the groups. The confidence level was taken as 95%.

RESULTS:

LYMPH NODE AND SPLEEN 0-3HR AND SWAP STIMULATED IL-5 PROLIFERATIVE RESPONSES:

Lymph node and spleen cells antigen stimulated IL-5 proliferative responses were tested. The lymph node cells stimulated by the antigens recorded significantly higher IL5 production compared to PZQ ($p<0.05$; Fig. 1A). Comparably, mice treated with either of the extracts showed significantly greater IL-5 responses to PZQ ($p<0.05$). However, the two crude extracts showed similar responses against the two antigens with no significant difference ($p>0.05$).

In the spleen cells, the responses indicated that all scenarios induced IL-5 production (Fig. 1B). There was an elevated proliferation of IL5 against the two antigens compared in PZQ treated group compared to the infected control with significant difference ($p<0.05$). The group *Carica papaya* aqueous, demonstrated significantly elevated levels of IL5 compared to the infected control ($p<0.05$) while the *Carica papaya* methanol group showed similar results with no significant difference ($p>0.05$)



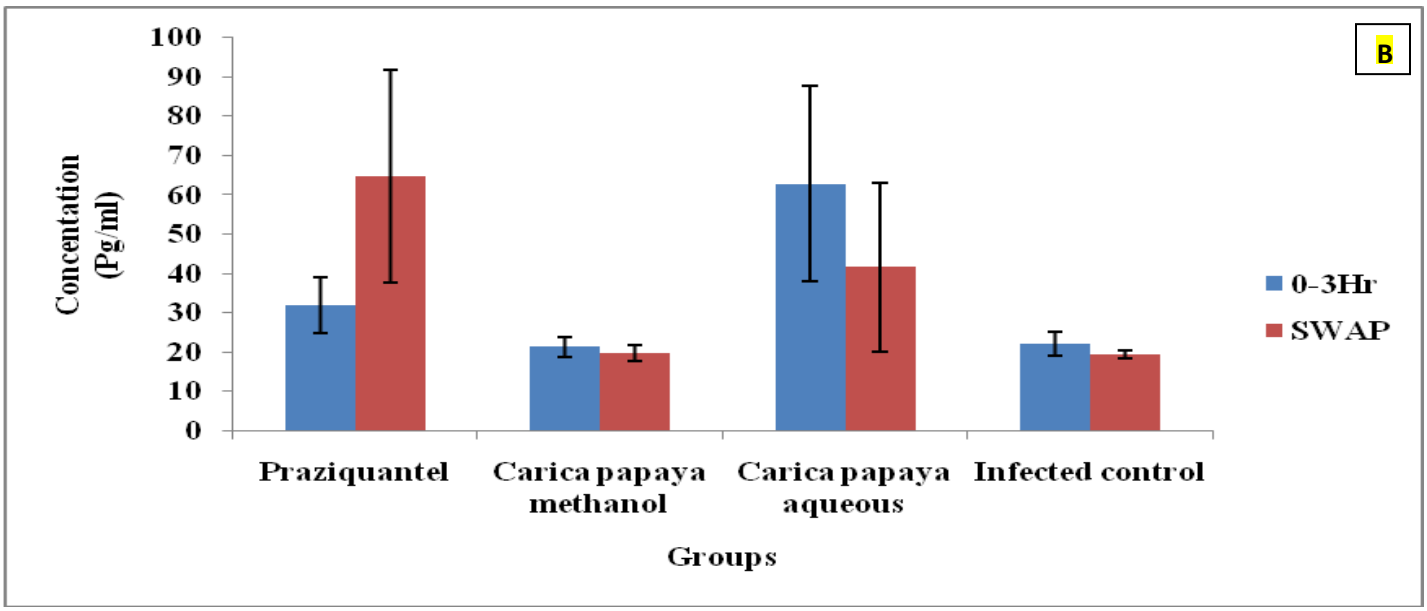


Figure No. 1: Graphical representation of the Lymph node [A] and spleen [B] 0-3hr and SWAP stimulated IL-5 proliferative responses. Lymph node and spleen cells were cultured at 3 x 10^5/ml in the presence of 10µg/ml per well of 0-3hr or SWAP and supernatants were harvested at 72hours.

LYMPH NODE AND SPLEEN 0-3HR AND SWAP STIMULATED IFN-Γ PROLIFERATIVE RESPONSES:

In general, cellular proliferative responses to Con A were greater than those observed for either *S. mansoni* or L. major antigens (data not shown). This is expected since Con A is a non-specific lymphocyte proliferation stimulator. When *S. mansoni* 0-3hr and SWAP antigens were individually used to stimulate lymph node and the spleen cells, IFN-γ and IL5 proliferative responses greater than the negative control were observed at the endpoint for cells from the infected control and all the treatments.

In the lymph node, similar IFN-γ responses were generally demonstrated for both the two antigens with no significant difference (P>0.05; Fig. 2A). The infected control and PZQ groups showed comparably significant diminished IFN-γ responses to 0-3hr and elevated responses to SWAP ($p<0.05$).

Proliferative responses against the antigens were also tested in lymphocytes obtained from the spleen. Responses from the infected control and other treatment groups were greater than the background at the end of the experimentation. Comparably, *Carica papaya* aqueous demonstrated diminished IFN-γ responses to *Carica papaya* methanol for both antigens with no significant difference (P>0.05; Fig.2B). Generally, IFN-γ responses stimulated by the SWAP antigen were higher compared to those stimulated by the 0-3hr antigen.

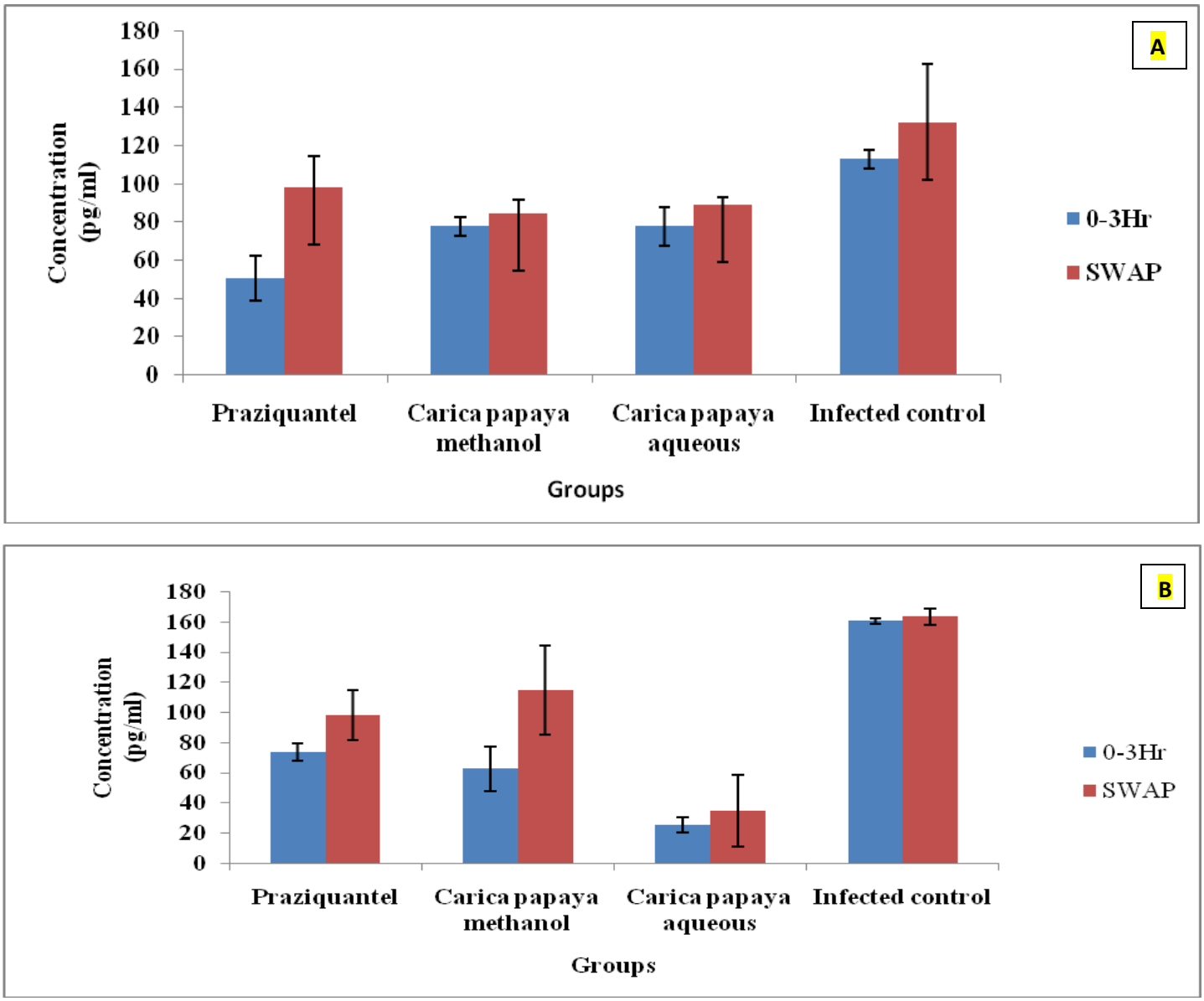


Figure No. 2: Graphical representation of the Lymph node [A] and spleen [B] 0-3hr and SWAP stimulated IFN-γ proliferative responses. Lymph node and spleen cells were cultured at 3 x 10^5/ml in the presence of 10µg/ml per well of 0-3hr or SWAP and supernatants were harvested at 72hours.

IgG RESPONSES:

Following infection, mice were tested for IgG responses to *S. mansoni* (0-3hr and SWAP) antigens in the fourth and sixth week following infection. Correlations were observed between 0-3hr and SWAP antigen specific IgG responses and resistance to infection before and after treatment in both the treatment and the control groups (Fig.3). The results obtained demonstrated expected rise in IgG level in response to 0-3hr and SWAP antigen as the infection progressed. In both groups, 0-3hr and SWAP IgG specific responses were similar from week four up the time the experiment was terminated at week 6 with no significant difference ($p>0.05$). Generally, the SWAP IgG responses were higher than 0-3hr.

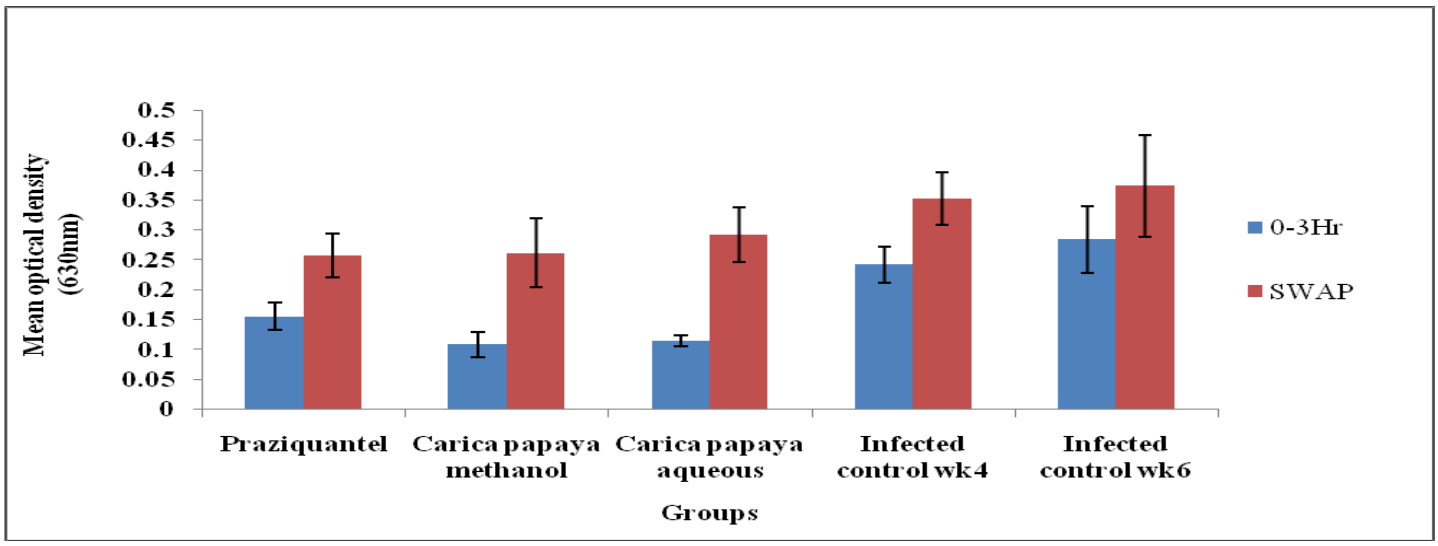


Figure No. 3: Graphical representation of the 0-3hr and SWAP antigen specific IgG responses of BALB/c infected with *S. mansoni* and treated with plant extracts. Each group represents the mean± SE of six observations.

PARASITOLOGICAL OUTCOMES: WORM RECOVERY AND MATURATION:

Worm recovery and reduction for each group was calculated as follows:

$$\text{Worm recovery} = \frac{(\text{Mean of total worms in experimental group})}{(\text{Mean of total worms in infected control})} X\ 100\%$$

$$\text{Worm maturation} = \frac{(\text{Number of worms recovered})}{(\text{Initial number of infecting cercariae})} X\ 100\%$$

The infected control group recorded higher worm recovery which represented a 16.7% maturation of penetrant cercariae. Comparatively, more worms were recovered in the group *Carica papaya* aqueous than *Carica papaya* methanol with no significant difference ($p>0.05$; Table 1). The mean worm recovery for the infected control was significantly higher compared to PZQ ($p<0.05$).

		Mean number of worms recovered per group (Mean ± SE)				
Group	Dose (mg/kg)	Total males	Total females	Total worms Mean±S.E	%worm recovery	%worm Reduction
Praziquantel	900x2	9.8 ± 0.72	4.2 ± 0.40	14.0 ± 0.86	33.5	66.5
Carica papaya methanol	300x2	15.3 ± 1.83	8.5 ± 2.10	23.8 ± 3.64*	56.9	43.06
Carica papaya aqueous	300x2	18.8± 2.23	10.7 ± 1.73	29.0 ± 3.51*	69.5	30.6
Infected control	0	24 ± 3.67	17.8 ± 1.12	41.8 ± 4.54	-	-

Table No. 1: Effect of *Carica papaya* on adult worm recovery.

Values are expressed as the mean ± SEM of six observations

*P<0.05 Statistical comparisons are made between: Control vs CPA and CPM groups

KEY: Dose=mg (plant extract/PZQ)/kg X number of doses

DISCUSSION:

Currently available drug regimens for treatment of schistosomiasis have some drawbacks. The potential for the development of resistance to PZQ was highlighted in 1995 by its apparently low efficacy, when used to treat a newly established focus of *S. mansoni* in Senegal[7]. During mass treatment, PZQ does not prevent re-infection in addition to it's high price hence a need for an alternative drug. In addition, the drug has two major administration drawbacks, the high dosage, and it's bitter and disgusting taste. In Egypt, some patients who received three doses of PZQ failed to get complete cure [9]. This study was therefore undertaken to assess antischistosomal properties of *Carica papaya* which has been reported as a novel agent.

In the present study, the percentage worm maturation revealed that a significant number matured and did not succumb to the effects of the extracts. Schistosomule develop into young adults and pair between 28 – 35 days post infection. In mouse model system, only about 20% of initial cercarial inoculum makes it to the adult stage. The greatest loss of larval stages occurs during the migration through the lungs with relatively smaller losses during migration through the skin[21]. 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.

In our study, treatment with a sinlge dose of the methanolic and aqueous seeds extract of *Carica papaya* showed appreciable anti-schistosomal activity in infected mice. So it seems that the extracts contained individual compound(s) responsible for the activity observed in vivo in amounts that could achieve relatively sufficient curative serum concentration in the mesenteric and portal vessels. The infected control group recorded high number of worms because most of the worms had matured and migrated to the mesenteries and were recovered during perfusion without any drug induced destruction

In studying the cytokine production profiles of spleen and lymph node derived lymphocytes, the host immune response to *S. mansoni* infection has been shown to be a T-cell dependent process. Classically, the host initially responds with a Th1 type response which has been shown to be directed against early stages of the parasite and to be important for the induction of the cell mediated protective immunity to *S mansoni*[21]. Accordingly, in this study, the infected control group demonstrated significantly higher IFN-γ responses than the treatments groups and PZQ in both the lymph node and spleen cells stimulated with both SWAP and 0-3Hr antigens. This is expected as IFN-γ is required in the initiation of granulomatous infection (cellular infiltration). The *Carica papaya* methanol group demonstrated similar responses to praziquantel especially for spleen cells responses. The IFN-γ response for SWAP was all time higher as compared to 0-3Hr antigen as it involves dealing with adult worms. Lymph node cells showed higher responses compared to spleen cells. This is expected because there is usually higher concentration of circulating antigens in the lymph nodes than spleen.

In this study, *Carica papaya* extracts induced production of IL-5. The higher levels of IL5 production by infected control, *Carica papaya* methanol, and *Carica papaya* aqueous in the lymph node and *Carica papaya* aqueous in the spleen could be as a result to non-specific schistosome antigens, which are not responsible for protection to schistosomes. These groups recorded higher worm recovery as compared to PZQ. This would translate to high levels of non-specific IgG, as suggested by high levels of IgG recorded in these groups.

Increased susceptibility to *schistosoma mansoni* infection has been associated with elevated adult worm specific IgG[22]. In this study, serum antibody analysis was done using cercarial 0-3Hr and soluble worm antigen preparation (SWAP) protein antigens. For both 0-3Hr and SWAP antigens, the IgG responses in infected control increased from week 4 to week 6. This increase is expected because worms are more mature at week 6 compared to week 4 hence more antigens were being released stimulating increased antibody response at week 6. Similar results have also been reported by Yole *et al.,* (1996b)[23]. There had also been no treatment in this group and there are no adult worms killed by extracts and hence there was continued release of antigens from ova and further stimulation of B- lymphocytes. These results also revealed that serum stimulated by 0-3hr antigen exhibited low IgG response as compared to SWAP. This is usually expected since the assay was done at the time when the parasites had developed into mature worms. What the 0-3hr antigen detected was shared antigens between the different stages of schistosomes. IgG response to 0-3Hr antigen could be lower because of the continuous change of the surface antigens of the schistosomules as they mature and also because of the effect of the host material which the worm covers itself with as a mechanism of evading immune response against self as it moves to its final destination.

The IgG production to SWAP antigens was high because of the consistency in antigens exposed on the surface due to maturity of the worm into adult worm. This variation in the level of IgG to Schistosome specific antigens shows that there is greater protective immunity to adult worm than to immature ones as high IgG level indicated increased immune protection. These results agree with the SWAP

antigen and IL-5 responses where IC had higher response than PZQ. The cytokine IL-5 is responsible for antibody production. This therefore means that, an increase in IL-5 also directly increases the production of antibodies.

CONCLUSION:

The methanolic and aqueous extracts of *Carica papaya* seeds have antischistosomal activity as they both record significantly reduced worm recovery in infected mice. This is an indication of their better antischistosomal property. Thus they can be used in the treatment of schistosomiasis. Hence further studies are required to know the exact mechanism of action and compounds responsible for antischistsomal effect.

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REFERENCES:

1. Jordan P, Gerald W, Roberts FS: Human Schistosomiasis. © CAB International Wallingford UK; 1993; pp.1-441.
2. Oliveira, G.; Rodrigues N.B., Romanha, A.J., Bahia, D. "Genome and Genomics of Schistosomes". *Canadian Journal of Zoology* 2004; 82 (2): 375–90. doi:10.1139/Z03-22
3. McKerrow, JH, SalterJ,. Invasion of skin by Schistosoma cercariae. Trends Parasitol; 2002: 18 N (5); 193-195.
4. Brindley P, A. Sher. The chemotherapeutic effect of praziquantel against *Schistosoma mansoni* is dependent on host antibody response. *J. Immunol* 1987; 139:215-22
5. PJ Brindley and A Sher. Sher. Immunological involvement in the efficacy of praziquantel. *Exp. Parasitol* 1990; 71:245-248
6. Gönnert, R. and Andrews, P. Praziquantel a new broad spectrum antischistosomal agents. *Z. Parasitenkd* 1977; 52: 129 – 150.
7. Stelma FF, Talla I, Sow S, Kongs A, Niang M, Polma K, Deelder AM Gryseels B. Efficacy and side effects of praziquantel in an epidemic focus of *S.mansoni*. *Am. J. Trop. Med. Hyg* 1995; 53: 167 - 170.
8. Dunne, D. W., A. E. Butterworth, A. J. C. Fulford, H. C. Kariuki, J. G. Langley, J. H. Ouma, A. Capron, R. Pierce, and R. Sturrock. Immunity after treatment of human schistosomiasis: association between IgE antibodies to adult worm antigens and resistance to re-infection. *Eur. J. Immunol* 1992; 22:1483-1494
9. Ismail MM, Metwally A, Farghaly A, Bruce J, Tao LF, Bennett JL. Characterization of isolates of *S. mansoni* from Egyptian villages that tolerate high dose of praziquantel. *Am. J. Trop. Med. Hyg* 1996; 55: 214 – 218.
10. Hammond JA, Fielding D, Bishop SC. Prospects for plant anthelmintics in tropical veterinary medicine. *Veterinary Research Communications* 1997; 21: 213 – 228.
11. Kokwaro, J. O. (1993). Medicinal Plants of East Africa, 2nd Edition, Kenya Literature Bureau, Nairobi, Kenya
12. WHO, 2002. WHO Traditional Medicine Strategy 2002-2005. World Health Organization, Geneva.
13. John Mokua Mose, Helen L. Kutima, Susy Muchika, Rebecca Waihenya, Dorcas S. Yole. The Evaluation of the Effects of an Aqueous and Methanol Extracts of *Solanum incanum* on *Schistosoma mansoni* Infected Mice. *Asian J. Pharm. Hea Sci* 2012; 2: 278-281.
14. Mohamed AM, Metwally NM, Mahamoud SS. Sativa seeds against *Schistosoma mansoni* different stages. *Mem. Inst. Oswaldo Cruz* 2005; 100: 205 – 211.
15. Sher, Z. A safe, effective herbal antischistosomal therapy derived from myrrh. *Am. J. Trop. Med. Hyg* 2001; 65: 700 – 704.
16. Ramalhoation-Pinto FJ, Gazinzinelli G, Howells RE, Mota-Santos TA, Figueiredo EA, Pellegrino J. Schistosoma mansoni: defined system for stepwise transformation of cercaria to schistosomule in vitro. Exp Parasitol. 1974;36:360–372. doi: 10.1016/0014-4894(74)90076-9
17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding. Analytical Biochemistry. 1976; 72: 248-254.
18. Smithers SR and Terry RJ. The infection of laboratory hosts with cercariae *of Schistosoma mansoni* and the recovery of the adult worms. *Parasitology.* 1965;55: 695-700.
19. Penina Njoki Muchirah, Dorcas Yole, Hellen Kutima, Rebecca Waihenya, Kennedy Muna Kuria and Mokua John. Determination of effective praziquantel dose in different mouse strains: BALB/c and Swiss mice in treatment of *Schistosoma mansoni.* Journal of Clinical

Immnunology and Immunopathology Research Vol. 4(2), pp. 12–21, March 2012

20. Yole DS, Pemberton R, Reid GDF and Wilson RA. Protective immunity to *Schistosoma mansoni* induced in the olive baboon Papio anubis by the irradiated cercaria vaccine. Parasitology. 1996; 112:37-46.

21. World Health Organization, 2002. Expert Committee. Prevention and Control of Schistosomiasis and Soil-Transmitted Helminthiasis. Geneva: WHO/CDS/2004.9

22. Gryzch JM, E. J. A. Cheever, Z. A. Caulada, P. Caspar, S. Hieny, F. A. Lewis, and A. Sher. Egg deposition is the major stimulus for the production of Th2 cytokines in murine *Schistosomiasis mansoni*. *J. Immunol* 1991; 146:1322-1327

23. Yole DS, Reid GDF and Wilson RA. Protection against *Schistosoma mansoni* and associated immune responses induced in the vervet monkey *Cercopithecus aethiops* by the irradiated cercariae vaccine. *American Journal of Tropical Medicine and Hygiene*.1996b; 54:265-270