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Antischistosomal Effects Of Solanum Incanum And Carica Papaya Crude Extracts On The Parasite Schistosoma Mansoni In Vivo And In Vitro

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Abstract

In schistosomiasis infection, the disease is managed by exposing the definitive host to a dose of Praziquantel. However, Praziquantelis 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. Antischistosomal effects of crude Solanum incanum and Carica papaya extracts were studied. Patterns on immune response, worm recovery, gross pathology in vivo and cercaricidal killing in vitro of Schistosoma mansoni was observed. In vivo
S. mansoni infections were treated with two doses of 150 mg/kg of Solanum incanum or Carica
papaya (methanol or aqueous) extracts and a treatment control of 450 mg/kg of Praziquantel. Various concentrations of plant extracts were used in cercaricidal assay. Carica papaya, showed highly reduced pathology, elevated immune responses and least time in destroying cercariae. On the other hand, S. incanum had the highest reduction in worm counts, similar to Praziquantel. Further studies are required to isolate the active compound(s) and determine mechanism(s) of their action.

INTRODUCTION

Schistosomiasis is a major disease of public health in humans, occurring in over 74 countries of the tropics and sub-tropics (WHO, 2010). It affects an estimated 207 million or more individuals and cause an estimated 500,000 deaths every year. Its current increased prevalence in many areas has numerous causes, including increased irrigation in areas with inadequate waste disposal and breakdown in public health infrastructure. The most common significant clinical effects of infection are intestinal and hepatic manifestations, which can result in serious illness or death (David et al., 2006). In Kenya, the infection is wide spread around Mwea irrigation scheme in Kirinyaga district, Machakos, Kitui, Taita Taveta and Nyanza (WHO, 2006).

The possible existence of S. mansoni isolate tolerance to Praziquantel has been reported in Senegal where the parasitological cure rate 12 weeks after treatment was as low as 18% (Berquist, et al., 2002). In regions of Egypt and Kenya where there has been heavy exposure to Praziquantel, there are reports of S. mansoni and S. haematobium resistance to treatment (Ross et al., 2002). This has necessitated the search for alternatives to praziquantel and other tools for control of schistosomiasis.

Plants are frequently discussed as possible sources of novel drugs, and in recent years they have been investigated as potential sources of antiparasitic agents including novel antischistosomal agents (Sher, 2001; Hagan et al., 2004). Crushed seeds of the plant Nigella sativa (Rununculaceae) were found to have antischistosomal activity against different stages (cercariae and juvenile) of S. mansoni (Mohamed et al., 2005). Artemisinin derivatives, now seen as important antimalarials, have been shown to have an effect on schistosomes. In China, artemether has been used successfully for acute S. japonicum infections in times of flood (Xiao et al., 2000). In this study, S. incanum and C. papaya methanol and aqueous extracts were assayed in vivo and in vitro for antischistosomal activities.

METHODOLOGY

HERBAL EXTRACTS

Solanum incanum roots and Carica papaya seeds were collected and placed in plastic bags. The plants were dried at room temperature for 2 months and crushed into tiny particles using Mekon Micromealer Single Phase and passed through a 0.5 mm mesh to standardize the particles. The ground plant material for each plant was divided into two equal portions and separately placed in two clean large bottles. Distilled water was added to one bottle holding each plant type until the sample was slightly submerged, after which it was left to soak for 72 h. The content was filtered; the process of soaking and filtering was repeated 3 times. The three filtrates were mixed in a container and freeze-dried using a freeze-drying machine (model FD-A made in Japan) for a month after which the aqueous extract was obtained in powder form.

Methanol was added to the other samples in the bottles until the samples were slightly submerged, soaked for 36 h and the content was filtered. The filtrate was placed in a round bottomed flask and fixed on a clean rotary vacuum evaporator (RE-100 Bibby, made in Japan). Temperature was set at 70°C, the mouth of the machine closed and power switched on. The machine was switched off when methanol stopped running from the distiller. The sample was then removed from the machine and further dried on a water bath until there was no evaporation of methanol (methanol extract).

PARASITE

Schistosoma mansoni isolate used in this study originated from infected baboons at the Institute of Primate Research (IPR), Karen, Nairobi and maintained in Biomphalaria pfeifferi snails collected from Kakuyuni in Kangundo. The snails were placed individually in each well of a 24-culture plate. Each snail was infected with 3-6 miracidia. The miracidia were left for 30 minutes to penetrate. The infected snails were placed in plastic tanks containing un-chlorinated water (snail water), sterilized sand and pebbles. Daphnea were included for aeration. The snails were fed on lettuce and maintained at the IPR Malacology laboratory. Four weeks post infection the snails were covered with a dark cloth to prevent shedding of cercariae. Five weeks after infection, snails were placed under strong light to induce shedding of cercariae for mice infection and cercaricidal assay.

DEFINITIVE HOST

BALB/c mice acquired from IPR Animal Resources Department were housed in cages, in groups of five per cage. They were maintained on a commercial diet and water ad libitum. The animals were under light/dark cycle of approximately 12 h/12 h at ambient temperature (20°C) and 50-60% relative humidity.

EXPERIMENTAL PROCEDURES

Mice were divided into six categories of 15 mice each, representing treatment and infected control groups infected with S. mansoni and an un-infected control group having 5 mice. The mice to be infected with

S. mansoni were anaesthetized using 0.02 ml Ketamine/xylazine mixture (ratio of 3:1). Each mouse received approximately 200 cercariae of S. mansoni through intact skin penetration by abdominal skin exposure using the ring method (Smithers and Terry, 1965). Treatment was done at week 4 post-infection with two doses two days apart. Each dose was 150 mg/kg body weight of the plant extracts. There were two groups for each plant extracts; aqueous and methanol. There were two control groups; one was treated with 450mg/kg body weight of Praziquantel (Farah et al., 2000), and the other was infected-untreated group.

SAMPLING TIME POINTS

Blood was obtained via heart puncture as follows; 5 naive mice (uninfected control) were sampled at wk 0; and 5 mice from each infected/ treated groups at wk 6. The blood was used to prepare serum for IgG ELISA. Five mice from all experimental groups and controls (PZQ and infected control) were perfused for worm recovery at week 6 and their livers were observed for gross pathology.

SCHISTOSOME SPECIFIC IGG ENZYME LINKED IMMUNOSORBENT ASSAY (IGG-ELISA)

Blood obtained from heart puncture was allowed to stand at room temperature for 3 h and incubated overnight at 4 °C. The clotted blood was centrifuged in a Microfuge (Sorvall RT 6000D made in Japan) at 2000 rpm for 20 minutes and sera retrieved. Nunc-Immuno™ plates (MaxiSorp ™ Surface) ELISA plates were coated overnight with 50 μl of 10 μg/ml of soluble worm antigen preparation (SWAP) or 18 hr schistosomule soluble antigen (SSP) diluted in bicarbonate buffer, pH 9.6 and incubated overnight at 4 ° C. The antigen was dispensed off on a blotting paper. The plates were washed six times using the washing buffer (0.05 % Tween 20 in PBS). This was followed by blocking of the non-specific binding sites with 100 μl 3% BSA in PBS and incubating at 37 ° C for 1 h. The plates were washed off unbound BSA six times with washing buffer. Diluted (1:200) serum samples (50 μl) was dispensed into each well in duplicates and incubated overnight at 4 ° C, and then washed as above. After washing the unbound serum, 50 μl of

1:2000 peroxidase conjugated rabbit anti-mouse IgG was dispensed into the wells and incubated for 1 h at 37°C. The unbound conjugate was washed off as before and

50 µl TMB micro well peroxidase substrate (Sure Blue ™ TMB) was added. The plates were incubated at 37 ° C in the dark for 30 minutes and optical density was read at 630 nm in an ELISA micro plate reader.

WORM BURDEN

At week 6 post-infection (2 weeks post-treatment), five mice from each group were perfused according to a modified method of Smithers and Terry (1965). Adult worm recovery was done according to the method described by Yole et al. (1996). Worm maturation was calculated using the following formula:

Figure 1

$$\text{Worm maturation} = \frac{\text{Number of worms recovered from infected control}}{\text{Initial number of infecting parasites}} \times 100\%$$

$$\text{Worm recovery} = \frac{\text{Number of worms recovered from treatments}}{\text{Number of worms recovered in Infected - untreated control}} \times 100\%$$

GROSS PATHOLOGY EXAMINATION

At week 6, gross pathology of the liver was observed in all the groups. The indices of comparison were; inflammation, adhesions and presence or absence of granulomas on the liver. The granulomas were categorized into none (no granuloma), few (1-3 granulomas), moderate (4-10 granulomas) and severe (>10 granulomas) per lobe.

CERCARICIDAL ASSAY

Two millilitre of each of plant extract (5 ug/ml, 15 ug/ml and 30/ml) was dispensed in a well of 24 cell culture plate containing an aliquote of 20 cercariae. Two replicates for each concentration was made. Each preparation was observed under a dissecting microscope for cercariae motility at the following time points: 5, 10, 20, 30, 45 and 60 minutes. Immobile cercariae were noted at every point; when all cercariae were immobile before 1 h, the experiment was terminated. At the end of each experiment, iodine was added for clarity in counting of the total number of cercariae as a confirmation of accuracy of the counting procedure.

ANALYSIS

Analysis of worm recovery and immunological data was performed using Student's t-test using computer excel programme and significance difference was defined as p<0.05. Gross pathology was noted by visual observation of liver tissues and cercaricidal assay was by enumerating the larvae under a microscope in percentages.

RESULTS

WORM MATURATION

The maturation level of the Kibwezi isolate of S. mansoni in BALB/c, maintained in snails from Kakuyuni. was 28.5%.

WORM RECOVERY

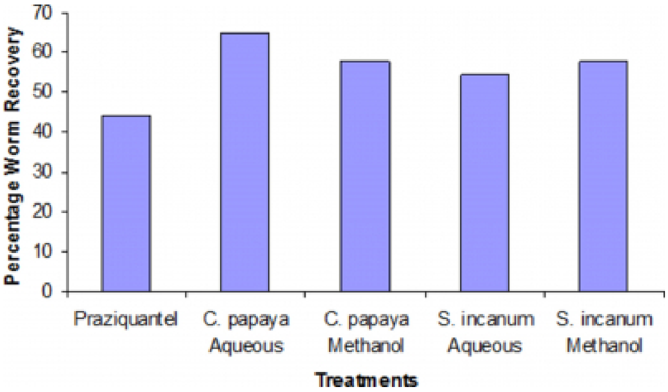
The mean number of S. mansoni worms recovered in the infected-untreated control, Praziquantel, C. papaya aqueous, C. papaya methanol, S. incanum aqueous and S. incanum methanol groups at week 6, was 57±1.3, 25±2.4, 37±1.8, 33±1.4, 31±2.1 and 33±3.4, respectively. Praziquantel had the lowest mean number of worm recovered while infected-untreated control had the highest. The percentage worm recovery for Praziquantel, C. papaya aqueous, C. papaya methanol, S. incanum aqueous and S. incanum methanol groups were as follows; 43.9%, 64.9%, 57.9%, 54.4%and 57.9% respectively (Fig 1).

Worms recovered from different groups were subjected to Student's t test to determine their significant difference in comparison with each other. Infected-untreated control was significantly different from PZQ control at p<0.001. All the four treatment groups were significantly different from infected-untreated control; C. papaya methanol and S. incanum aqueous at p<0.001, S. incanum methanol at p<0.01 and C. papaya aqueous at p<0.05.

There was a significant difference between three treatment groups and PZQ: C. papaya methanol and S. incanum aqueous, p<0.0l and C. papaya aqueous p<0.05. However, there was no significant difference between S. incanum methanol and PZQ, p>0.05. Statistically this shows that S. incanum methanol and PZQ had similar worm recoveries.

Fig 1: Percentage Worm Recovery in Treatments

Figure 2



GROSS PATHOLOGY

In all mice in the six groups, all liver were inflamed and had adhesions. In infected-untreated control group, most of the mice had moderate granuloma while two mice had severe granuloma. Praziquantel group had only one mouse with few granulomas and the rest had no granuloma. In C. papaya aqueous treatment group, 4 out of 5 mice had few granulomas and only one mouse had no granuloma. In C. papaya methanol 3 mice had a few granulomas and 2 mice did not have any granuloma. In S. incanum aqueous and methanol groups, four of the mice had few granuloma and only one mouse had moderate granuloma. Generally the lowest granuloma levels were observed in Praziquantel group, while the infected control had the highest granuloma levels. The extract treatment groups' granuloma levels lay in between the controls, C. papaya being second best to PZQ as S. incanum followed.

IMMUNOGLOBULIN G RESPONSE TO SWAP AND SSP-SPECIFIC ANTIGEN

IgG responses were analyzed in seru collected from naive group, infected-untreated control and from treatment groups week-2 post-treatment (6 weeks post-infection). The results are shown in Feig 2.

IG response to SWAP antigen in infected-untreated control at week 6 was not very high (O.D 0.319). Praziquantel had a high IgG response in week 6 (O.D 0.352) which was significantly higher than the response in infected-untreated control ($p<0.05$).

In C. papaya aqueous, the IgG level (O.D 0.452) was higher than that of PZQ (O.D 0.352) and infected-untreated control (O.D 0.319) at week 6. However in C. papaya methanol treatment, IgG response was lower (O.D 0.367) at week 6 in comparison to the aqueous treatment but was slightly higher than that of PZQ and infected-untreated control. The IgG response in both C. papaya methanol and aqueous was significantly higher than PZQ ($p<0.05$).

Solanum incanum aqueous group had IgG level of O.D 0.331, which was not significantly different from PZQ ($p>0.05$) while S. incanum methanol group had the lowest IgG response, O.D 0.218, at week 6. The IgG response in the aqueous and methanol treatments was significantly lower than that of PZQ ($p<0.05$).

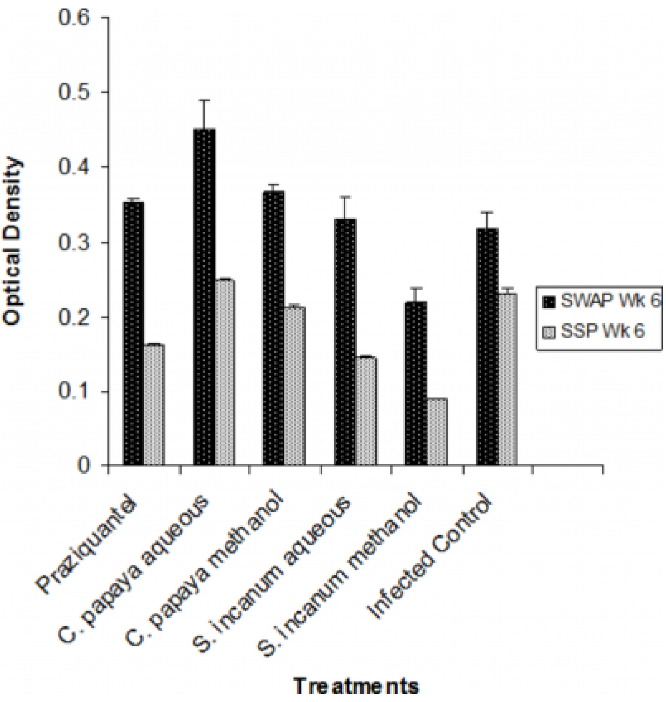
In assay for SSP-specific IgG responses, infected-untreated control group had higher IgG responses (O.D 232) than Praziquantel treatment (O.D 0.162). This difference was significant ($p<0.05$).

In C. papaya aqueous, IgG level (O.D 0.247) was significantly higher than that of C. papaya methanol (O.D 0.213; $p<0.05$). The responses in S. incanum aqueous group (O.D 0.144) was significantly higher than that of S. incanum methanol group (O.D 0.090; $p<0.05$). C. papaya had significantly higher IgG response compared to S. incanum extract treatments ($p<0.05$). C. papaya methanol ($p<0.05$), S. incanum aqueous and methanol groups ($p<0.01$) had significantly lower responses than infected-untreated control.

IgG responses to SSP antigen were lower in relation to IgG responses to SWAP antigens in all the treatments and controls

Figure 3

Fig 2: IgG Responses to SWAP and SSP Antigen in Schistosome Infected Mice



KEY

Wk = week

SWAP = soluble worm antigen preparation

SSP =18 hr schistosomule soluble antigen

CERCARICIDAL ASSAY

The cercaricidal effect of the plant extracts is shown in Table 1. The results are the means of duplicate observations of lowest (5 µg/ml), moderate (15 µg/ml) and maximum (30 µg/ml) concentrations of the plant extracts at different time intervals.

In both aqueous and methanol C. papaya treatments, at concentrations of 5 µg/ml and at the 5 th minute, about a quarter of the cercariae was dead; at the 10 th minute about a half of the cercariae were dead and at the 20 th minute all the cercariae were dead. The two treatments had similar strength in killing the larvae worms at the lowest concentrations. However there was great difference in the effects of both methanol and aqueous of C. papaya extracts at the moderate (15 µg/ml) and maximum (30 µg/ml) concentrations. At the 5 th minute; 15 µg/ml and 30 µg/ml concentrations of C. papaya methanol group, all the cercariae had lysed and could not be enumerated. In C. papaya aqueous at, 15 µg/ml and 30 µg/ml about three quarters and slightly over three quarters of the cercariae were dead, respectively, at the 5 th minute. At the 10 th minute all cercariae at the same concentrations were dead. The methanol extracts at increased concentrations (15 and 30 µg/ml) showed a stronger ability in killing the cercariae than the aqueous ones, as it led to lysis of the cercariae.

In S. incanum treatments at concentrations of 5 µg/ml at the 5 th minute, about a quarter of cercariae were dead in aqueous treatment, and about a third of the cercariae were dead in methanol group. At the 10 th minute in both aqueous and methanol extracts, about a half of the cercariae were dead while at the 20 th minute, all the cercariae were dead in both concentrations. The killing strength of aqueous and methanol extracts was almost the same at the lowest concentrations. At 15 µg/ml, 5 th minute, in aqueous group, there were over three quarter deaths of cercariae and 100 % death was realised at the 10 th minute. However at the same concerntration, all cercariae were dead in the methanol treatment at the 5th minute.

At a concentration of 30 µg/ml of S. incanum aqueous treatment, all the cercariae were dead and not lysed at the 5 th minute, while in the methanol treatment; cercariae had been lysed at the same duration and concentration. However, no cercariea was dead after one hour in aqueous control.

Figure 4

Table 1: Effects of Plant Extracts on Cercariae at different concentrations

Extracts	Concentration in µg ml	5 minute reaction in %	10 minute reaction in %	20 minute reaction in %
C. papaya methanol	5	23.6	46	100
C. papaya methanol	15	*_	_	_
C. papaya methanol	30	*_	_	_
C. papaya aqueous	5	26.7	55.55	100
C. papaya aqueous	15	71.9	100	_
C. papaya aqueous	30	85.35	100	_
S. incanum methanol	5	36.3	50.2	100
S. incanum methanol	15	100	_	_
S. incanum methanol	30	*_	_	_
S. incanum aqueous	5	25.4	49.7	100
S. incanum aqueous	15	83.9	100	_
S. incanum aqueous	30	100	_	_
Aqueous control	0	0	0	0

KEY

*_ cercariae were dead and lysed
_ Observation stopped

DISCUSSION

This study compared the efficacy of S. incanum aqueous/methanol and C. papaya aqueous/methanol plant extracts on S. mansoni. The efficacy was assessed based on the worm recovery, gross pathology and IgG immune responses to SSP and SWAP antigens. Cercaricidal assay was also done to determine the survival rate of cerceriae in various concentrations of different extracts of the two plants.

Kibwezi S. mansoni isolate maintained in Kakuyuni snails used in this study had worm maturation of 28.5% in BALB/c mice. A worm maturation of 12% was obtained in Golden hamsters-Mesocricetus auratus infected with S. haematobium Kijiwe isolate (Njoroge et al., 2007). Comparison of these two isolates of different species in different models shows that the Kibwezi isolate worm maturation is higher than the Kijiwe isolate.

The infected-untreated group had the highest worm burden (57±1.3) while Praziquantel had the lowest worm burden (25±2). Solanum incanum (33±3) methanol and C. papaya methanol (33±1) had the similar effect on worm reduction. Solanum incanum aqueous had the lowest worm recovery (31±2), which was similar to Praziquantel, therefore was the most efficacious treatment. On the other hand, C. papaya aqueous had the highest worm recovery (37±1) of the extracts treatments, therefore it was the least efficacious. The lowest worm burden in Praziquantel group was expected and can be attributed to the fact that Praziquantel has good efficacy against the adult S. mansoni worm (Utzinger and Keiser, 2004).

Gross pathological observations revealed that the livers of all the mice were inflamed and had adhesions, a manifestation of an infection. Granuloma formation in the liver was the worst in infected-untreated control group while Praziquantel had the lowest granuloma formation level. Although Praziquantel is the most efficacious drug (Utzinger and Keiser, 2004), some mice under this treatment had granulomas. This can be linked to the fact that some parasites may have delayed to mature thus escaped the effect of Praziquantel at the time of its administration. This is because Praziquantel drug has a short half-life of 1–1.5 hours (Ross et al., 2002) and it is not effective against schistosomules. C. papaya methanol and aqueous had a similar and lower granuloma formation compared to Praziquantel.S. incanum methanol and aqueous had similar but worse granuloma level in relation to C. papaya groups. This shows that C. papaya extracts reduced granuloma more compared to S. incanum extracts.

Infected-untreated control had high IgG responses to both SWAP and SSP antigens. 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 (Njoroge et al., 2007). This high IgG level did not confer protective immunity in infected-untreated control as demonstrated by the highest number of worm recovery and the worst pathology observed in this group.

The IgG responses in Praziquantel for both antigens 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.

Carica papaya aqueous had the highest IgG response to both SWAP and SSP antigens among the plant extracts. However, this did not translate to reduced worm numbers, implying non-specific stimulation of the immune response towards the killing of the parasites. However, there was reduction in granuloma formation pointing to an immune response, which either sterilized the females that they could not lay eggs, or reduced the granulomas. 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 Mojica-Heshaw et al. (2003) 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.

Solanum incanum aqueous had similar IgG responses to Praziquantel. Interestingly, its worm reduction was also similar to that of PZQ. A high IgG response signifies a high protective immunity of the treatment (Kanyugo et al., 2009). However, the gross pathology was worse than that of PZQ. S. incanum methanol,which had the lowest immune response, had a similar pathology to the aqueous extract. The methanol group was not adequately protected in terms of both worm reduction and also pathology.

Generally, in this assay, IgG responses to SWAP in all the 6 treatments were higher, compared to responses in SSP. The low IgG responses to SSP antigens could be due to reduced schistosomule antigens as the worms matured.

C. papaya methanol took the shortest time to kill cercariae compared to C. papaya aqueous. Similar trend was seen in S. incanum methanol in relation to the aqueous group. However, S. incanum methanol was less efficacious compared to C. papaya methanol group. The pattern was also different in aqueous groups; C. papaya aqueous was less efficacious compared to S. incanum aqueous. The maximum duration for the destruction of the cercariae in the four treatments; both aqueous and methanol treatments of C. papaya and S. incanum 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 (Jordan et al., 1993). A few cercariae can make this journey within five minutes (McKerrow and Salter, 2002) 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.

CONCLUSION

The two plants extracts demonstrated efficacy to S. mansoni infection at different direction: C. papaya, showed greater ability in reducing pathology, elevated immune responses and shortest time in destroying cercariae while S. incanum showed more strength in reducing worm number in the infected mice.

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References

r-0. Berquist NR Sherbiny ACM, Barahat R, Olds R: Blueprint for Schistosomiasis vaccine development. Acta Trop; 2002; 82: 183–192.
r-1. David R, Fenwick A, Vaughan S: Implementation of Human Schistosomiasis Control: Challenges and prospects. Adv Parasitol; 2006; 61:567-622.
r-2. Farah IO, Nyindo M, King CI, Hau J: Hepatic granulomatous responses to Schistosoma mansoni eggs in BALB/c mice and Olive baboons (Papio cynocephalus anubuis). J Comp Pathol; 2000; 133: 7 – 14.
r-3. Hagan P, Christopher C, Appleton G, Coles C, John R, Kusel, Louis–Albert Tchuem Tchente': Schistosomiasis Control: Keep taking the tablets. Trends Parasitol; 2004; 20: 92–97.
r-4. Jordan P, Gerald W, Roberts FS: Human Schistosomiasis. © CAB International Wallingford UK; 1993; pp.1-441.
r-5. Kanyugo MS, Ozwara H, Mutahi W, Yole DS: Parasitological and Immunopathological Responses Balb/C Mice with Concomitant Schistosoma mansoni and Plasmodium berghei Infections. The Internet Journal of Tropical Medicine; 2009; 5(2).
r-6. McKerrow, JH, SalterJ,. Invasion of skin by Schistosoma cercariae. Trends Parasitol; 2002: 18 N (5); 193-195.
r-7. Mohamed AM, Metwali NM, Mahamoud SS: Sativa seeds against Schistosoma mansoni different stages. Mem Inst Oswaldo Cruz; 2005; 100: 205–211.
r-8. Mojica-Henshaw MP, Fransisco AD, Guzman DF, Tigno XT: Possible immunomodulatory actions of Carica papaya seed extract. Clinical Hemorheol Microcirc; 2003; 29 (3-4):219-29.
r-9. Njoroge VK, Nyundo F, Limo M, Yole DS: A comparative study of multiple versus single infection doses of Schistosoma haematobium in Golden hamsters (Mesocricetus auratus). Afr J Health Sci; 2007; 4: 187-194.
r-10. Ross APG, Bartley PB, Sleigh AC, Olds, GR, Li Y, Williams GM, McManus DP: Schistosomiasis. 1212. N Engl J Med; 2002; 346: 16.
r-11. Sher Z: A safe, effective herbal antischistosomal therapy derived from myrrh. Am J Trop Med Hyg; 2001; 65: 700 –704.
r-12. Smithers SR, Terry RJ: The infection of the laboratory hosts with cercariae of Schistosoma mansoni and the recovery of the adult worms. Parasitology; 1965;55: 695-700.
r-13. Utzinger J, Keiser J: Schistosomiasis and soil-transmitted helminthiasis: common drugs for treatment and control. Expert Opinion Pharmacother; 2004; 5: 263–285.
r-14. World Health Organization: Schistosomiasis and Soil Transmitted Helminths Country Profile. World Health Assembly Resolution 54; 2006.
r-15. World Health Organization: Schistosomiasis Fact Sheet No115; www.who.int/mediacentre/factsheets/fs115/en/index.html; Updated February 2010.
r-16. Xiao SH, Chollet J, Weiss NA, Berquist RN, Tanner M: Preventive effect of artmether in experimental animals infected with Schistosoma mansoni. Parasitol Int; 2000: 49: 19–24.
r-17. Yole DS, Pemberton R, Reid GD, Wilson RA; Protective immunity to Schistosoma mansoni induced in the olive baboon, Papio anubis, by the irradiated cercariae vaccine. Parasitology; 1996; 112: 37-46.

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