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Antischistosomal activity of green tea (Camellia sinensis) against female unisexual infection of murine Schistosoma mansoni

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Abstract: The schistosomicidal action of green tea against female *Schistosoma mansoni* unisexual infection was investigated. The effects of green tea on the infection were assessed by two parameters, worm recovery and histopathological changes in the liver of the host. The results revealed that the worm burden was fewer in mice treated with green tea than in non-treated mice. Hepatic histological examination of infected non-treated mice revealed that the unisexual female infection induces hepatic lobular disorganization, cytoplasmic vacuolation of the hepatic cells, Kupffer cells hypertrophy and variable degree of necrosis. In infected mice treated with green tea, the ordinary liver lobular architecture was relatively restored; most hepatocytes were unharmed and fewer cells appeared vacuolated.

Keywords: Schistosomicidal, green tea, unisexual, histopathology

1. INTRODUCTION

Despite of strenuous control efforts, schistosomiasis is a tropical disease that ranks with malaria and tuberculosis as a major source of morbidity affecting approximately 210 million people in 76 countries of the world (Steinmann et al., 2006). In sub-Saharan Africa alone, approximately 280,000 deaths per annum are attributable to schistosomiasis[1]. The repeated chemotherapy of schistosomiasis in endemic areas has resulted in the emergence of drug-resistant schistosome strains[2-3]. The development of such resistance has drawn the attention of many authors to alternative drugs. Many medicinal plants such as plant quinghao *Artemisia anmia*[4], garlic *Allium sativum*[5-6], wild carrots *Daucus carota*[7], jungle weed *Combretum* sp.[8], myrrh *Commiphora molmol* [9], ginger *Zingiber officinale*[10-12], hound's berry or night shade *Solanum nigrum*[13] and the crude oil of black-seed *Nigella sativa*[14-17] were studied to investigate their antischistosomal potency and found to be effective.

Green tea (*Camellia sinensis*) is used for several medical purposes and its activities have been observed in various experimental models[18]. This plant contains a number of polyphenolic compounds, collectively termed catechines, epicatechin gallate, epigallocatechain gallate (EGCG). EGCG accounts approximately 50% of total amount of catechains; these compounds belong to flavan-3-ols family[19-21]. These compounds are known to have a broad spectrum of biological activities such as antioxidant and antiviral[22], anticancer [19-20], antibacterial[23], antifungal[24], anticoccidial[25-26], antitoxoplasmal[27], antitrypanosomal[21], antinematodial[28] and antihelminthic[26]. Moreover, the molluscicidal and larvicidal activities of green tea against intermediate host and free larvae of *S.mansoni* and *S.haematobium* were demonstrated[29].

Many studies was undertaken to evaluate the effect of unisexual infection on murine schistosomiasis model at different levels including histopathological investigations[30-33], resistance of mice to secondary schistosome infection[34], enzymes activities[35] and worm development[36].

Up to our knowledge the antischistosomal activity of green tea against unisexual or bisexual infections of schistosomes have been not previously studied. This study was undertaken to evaluate the antischistosomal activity of green tea against female unisexual infection of *Schistosoma mansoni* in albino mice by assessment of two parameters: worm recovery and histopathological changes in the liver of the host.

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2. MATERIALS AND METHODS

Source of parasite and host: *Biomphalaria alexandrina* snails shedding unisexual female *Schistosoma mansoni* cercariae were supplied by the Schistosome Biological Supply Program (SBSP) at Theodor Bilharz Research
Host infection: Sixteen BALB/C mice were exposed to 100±10 unisexual female *S. mansoni* cercariae per mouse by the tail immersion method, modified by Oliver and Stirewalt[37].
Treatment: Green tea was extracted using the method described by Hamden *et al.*[38] with few modifications.

Briefly, three grams of green tea were covered with 100ml boiling water for five minutes; the resulting extract was passed through filter paper to free the extract from insoluble material. This solution representing 3% was given orally to eight infected mice as sole source of drinking water from the end of the 4th week to the end of 10th week post-infection. Eight infected untreated mice were allowed to drink normal water. All mice were sacrificed at the end of 10th week post-infection.
Worm recovery: The recovery of *S. mansoni* worms from the hepatic portal system and mesenteric veins of sacrificed mice was done by the perfusion technique described by Smithers and Terry[39].
Histological preparations: Hepatic tissues of infected, non-treated and infected, treated mice were fixed in aqueous Bouin's solution. Specimens were dehydrated through ascending grades of alcholol, cleared in terpineol and finally embedded in paraffin wax. 5µm thick sections were taken by using a rotatory microtome. Sections were affixed on clean slides, deparafinized by xylol, hydrated through descending grades of alcholol and stained by Mayer's haematoxylin and 1% aqueous eosin. Sections were dehydrated in an ascending series of ethanol, cleared in xylol and finally mounted in Canada balsam and examined by research light microscope.

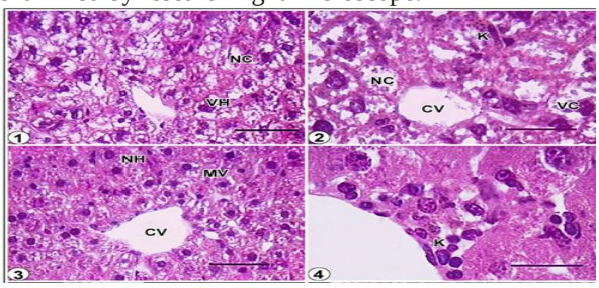

Figure 1. Photomicrograph of liver section of infected, non treated mouse revealing the disordered hepatic strands, vacuolated hepatocytes (VH) and necroticcells (NC). Bar=50µm. 2. Photomicrograph of liver section of infected, non treated mouse showing vacuolated cell (VC), necrotic cells (NC), Kupffer cells engulfed parasite pigments (K) and central vein (CV). Bar=50µm. 3. Photomicrograph of liver section of infected and treated mouse presenting restored ordinary liver lobular architecture, central vein (CV), unharmed hepatocytes (NH) and some moderately vacuolated hepatocytes (MV). Bar=50µm. 4. Photomicrograph of liver section of infected and treated mouse showing Kupffer cell loaded with parasite pigments (K). Bar=50µm

Institute, Imbaba, Giza, Egypt. Sixteen adult male albino mice of BALB/C strain, weighing 18-22g for each, were obtained from the animal house of Biology Department, Faculty of Science, King Khaled University, Abha, Saudi Arabia. The animals were given access to water and standard diet and were monitored daily for health status.
Statistical analysis: Results were subjected to Student's t-test using SPSS program version 8 to determine the significance of data.

3. RESULTS AND DISCUSSION

Worm recovery: The mean number of *S. mansoni* female worms recovered from treated group was (8.75±0.12) which is lower than that in the infected untreated group (15.25±0.18) and the difference between them is significant (P<0. 01).
Histopathology: Concerned with histopathological changes, no granulomatus reaction was observed in treated or non-treated infected mice due to the absence of deposited eggs in the tissue. However, in infected non-treated mice a high

degree of disorganization in the hepatic lobular structure was observed, as no liver cords could be followed. Schistosomal pigments egested by worms were deposited in the hypertrophied Kupffer cells. Furthermore, hepatocytes were obviously altered. Most cells showed an advanced degree of injury symptomized by cytoplasmic vacuolation. Some of these cells became distinctly necrotic. Nuclei of these injured liver cells became pleomorphic and pyknotic, as well as some of them appeared remarkably karyolysed (Figs.1-2). In infected mice treated with green tea, the ordinary liver lobular architecture was relatively restored. Most hepatocytes were unharmed, fewer cells appeared vacuolated. However, Kupffer cells were still loaded with schistosomal pigments (Figs.3-4).

Reduction in worm recovery due to green tea treatment was observed in the present study. Such observation

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was correlated with that of Molan *et al.*[28] who investigated anti-parasite activity of green tea components against the sheep nematodes, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* as evidenced by their ability to inactivate the infective larvae and inhibit their migration. The worm recovery reduction may be due to the killing effects of green tea against the schistosome parasite; this suggestion is supported by the observation of Paveto *et al.*[21] who reported that the green tea catechins had a lytic effects
against trypomastigote and amastigote forms of *Trypanosoma cruzi.*
In the current work the typical granulomatus reaction to schistosomiasis mansoni on the liver was not noted due to the absence of deposited eggs in the tissues, this observation correlated with that of Khalil and Mansour[36] on

their studies on worm development in hamsters infected with unisex and cross-mated *S. mansoni* and *S. haematobium*; they reported that *S.mansoni* females were stunted and partially mature but did not contain eggs. However, in the present study, other histological alterations were observed in the liver of infected non-treated mice including hepatic lobular disorganization, cytoplasmic vacuolation of the hepatic cells, Kupffer cells hypertrophy and variable degree of necrosis. These alterations, in absence of any eggs, may be due to antigenic substances secreted by female *S. mansoni.* This suggestion is supported by Boissier *et al.*[33] who stated that the digestive enzymes that are present in the gut of adult worms and are regurgitated into the blood constitute an important source of parasite antigens available to the immune system. These antigens are known to be highly immunogenic[40]. Comparable results were introduced by Baki *et al.*[32] who declared that in mice experimentally infected with male *S. mansoni*, from the 25th week post-infection, a diffuse fibrosis affected the main branches of the portal vascular system of the liver following the host inflammatory reaction, associated with the proliferation of myofibroblasts *in situ*. They suggested that antigenic substances secreted by

adult schistosomes, in the absence of eggs, might initiate periportal and perisinusoidal fibrous reaction. Boissier *et al.*[33] reported that, in unisexual infection, male schistosomes induced a stronger inflammatory response compared to female.

In the present study, hepatic histological examination of infected mice treated with green tea revealed a remarkable histological recovery. This may be perhaps because of the role of green tea as an anti-inflammatory, anti-hepatic fibrosis and activator of hepatic detoxification enzymes. Since, Nam *et al.* [41] cited that tea polyphenols have been shown to inhibit proteasome function, thereby terminating inflammation. Human and animal studies have demonstrated EGCG's ability to block inflammatory responses to ultraviolet A and B radiation as well as significantly inhibiting the neutrophil migration that occurs during the inflammatory process[42-43]. Kim *et al.*[44] examined the protective effect of green tea extract on hepatic fibrosis *in vitro* and *in vivo* in dimethylnitrosamine (DMN)-induced rats. They concluded that green tea administration can effectively improve liver fibrosis caused by DMN, and may be used as a therapeutic option and preventive measure against hepatic fibrosis.

4. CONCLUSION

In conclusion, it is obvious that green tea had schistosmicidal effects upon schistosomiasis mansoni in albino mice, therefore further studies will be done to evaluate the effect of green tea upon the bisexual infection of *S. mansoni* as well as identifying the appropriate dosage and fractions that must be used for treatment of infection at the different stages.

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