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Full Length Research Paper

Potential Effect of Camphor Oil as a Local Cercaricidal Agent

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Objectives: - The aim of this study is to clarify the deleterious effects of topical Camphor oil administration alone, in experimental *Schistosoma mansoni* infection. It is a trial to elucidate the stigmata left upon using this compound upon worm and tissue egg loads, together with oogram pattern in experimental murine schistosomiasis mansoni.

Abstract: - In this study, a group of 30 Swiss Albino mice was used. This group was further subdivided into three small subgroups: - Subgroup I: - constituted control infected untreated mice. Infection was done by dermal inoculation of 80 *Schistosoma mansoni* cercariae per mouse through a ring, after shaving the abdominal skin. Subgroup II: - abdominally shaved mice wiped with 100 µl per mouse Camphor oil, then infected 10 minutes later with 80 *Schistosoma mansoni* cercariae.

Subgroup III: - abdominally shaved mice wiped with 100 µl per mouse Camphor oil, then infected immediately after with 80 *Schistosoma mansoni* cercariae.

All animals were sacrificed 6 weeks post infection. Mice given Camphor oil, revealed a complete reduction (100 %) in the total worm recovery, when compared to the respective untreated controls (23±2.56). This value was the same when Camphor oil was spread 10 minutes before infection, and immediately after infection with 80 *Schistosoma mansoni* cercariae. Again, the former group revealed complete disappearance of tissue egg load (both hepatic and intestinal, and vanishing of eggs in the oogram compared to the latter group. as well as healthy hepatic parenchyma.

Keywords: Camphor oil, local cercaricidal, experimental schistosomiasis mansoni, mature worms, tissue egg load oogram and. granuloma measurement.

INTRODUCTION

In the past decade, medical science has made many strides towards the understanding of the pathogenesis of schistosomiasis. Non-immune travelers visiting endemic areas, are at high risk of acquiring the disease and disseminating the parasite to non-endemic areas (Sabri et al .2006). Despite tremendous innovation in the methods used to derive new antischistosomal compounds, the prevailing methods for testing them have remained essentially the same for decades. As basic

science has blossomed with the advent of praziquantel as a potent schistosomicidal compound, we should abandon the feeling that the perfect drug has been found once for all (Sabri et al .2006). Consequently, apart from parenteral chemotherapy, topical prophylactic measures are resorted to, in order to prevent and damp down the drastic and deleterious effects of the disease. Since skin is the only route of entry for this parasite into humans, intervention that prevents entry of this parasite into the skin, should control the infection (Stirewalt and Dorsey, 1974). Previously, Sabri et al (2006) tested the effect of topical Cedar wood oil administration alone and/or in

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combination with the solvent, DMSO (Dimethyl Sulphoxide), and Propolis (a natural bee wax extracted by bee from tree exudates) in experimental schistosomiasis mansoni. The authors deduced that Cedar wood oil could be successfully used in conjunction with DMSO and Propolis as a topical prophylactic agent in experimental schistosomiasis mansoni infection later on, Abdel Aziz et al (2011) tested the in vitro anti-Schistosomal activity of *Plectranthus terniflorus* on miracidium, cercariae, schistosomulae stages of *Schistosoma mansoni*. They stated that the methanolic extract of this plant (vatke), showed moderate anti-cercarial and anti-schistosomular activity with calculated Lc 50 12.29 and 17.39, respectively compared to lower anti miracidium activity with Lc 50 24.37 mg/100ml. Later on, Bagalwa et al (2015) tried the cercaricidal activity of a mixed solamargine (1) & B solamargine (2) solution against *Schistosoma mansoni* cercariae. This study could be promising in endemic areas like Egypt where indigenous or newly coming foreigners are exposed to unexpected infection while rowing or fishing in the Nile River.

MATERIAL AND METHODS

In this study, a group of 30 Swiss Albino mice was used. This group was further subdivided into three small subgroups:-

Subgroup I: - Constituted control infected untreated mice. Infection was done by dermal inoculation of 80 *Schistosoma mansoni* cercariae per mouse through a ring, after shaving the abdominal skin.

Subgroup II: - Abdominally shaved mice wiped with 100 µl per mouse Camphor oil, then infected 10 minutes later with 80 *Schistosoma mansoni* cercariae

Subgroup III: - abdominally shaved mice wiped with 100 µl per mouse Camphor oil, then infected immediately after with 80 *Schistosoma mansoni* cercariae. All animals were sacrificed 6 weeks post infection.

RESULTS

Mice given Camphor oil, revealed a complete reduction (100 %) in the total worm recovery, when compared to the respective untreated controls (23±2.56). This value was the same when Camphor oil was spread 10 minutes before infection, and immediately after infection with 80 *Schistosoma mansoni* cercariae.

Again, the former group revealed complete disappearance of tissue egg load (both hepatic and intestinal), and vanishing of eggs in the oogram compared to the latter group.

DISCUSSION

From this study, it could be concluded that local application of Camphor oil, exerts a prominent prophylactic effect in experimental schistosomiasis mansoni infection. This is evident by the complete disappearance of mature worms recovered at perfusion, in both infected treated groups (whether given topical Camphor oil immediately before infection, or 10 minutes before),

Again, this compound caused vanishing of the oogram pattern and tissue egg load (both hepatic and intestinal). This goes with the previous assumption of Pellegrino et al (1962). They stated that a dose level of the drug was considered to have a definitive action against *Schistosoma mansoni* when the oogram showed 50% or more mature eggs and absence of one or more stages of immature eggs.

Previously Xiaonong et al (1993) studied the effects of Niclosamide and Eucalyptus camaldulensis on *Biomphalaria glabrata* the snail intermediate host of *Schistosoma mansoni*. The authors observed histological changes in the intestines, digestive glands & ovotestes of the molluscicides treated snails. Many workers studied the components of essential oil from the leaves of Eucalyptus spp. Xiaonong et al (1993) deduced that the methanolic extract of Eucalyptus camaldulensis leaves is one of the promising molluscicides, at least against *Biomphalaria glabrata*.

Later on, Farooq et al (2007) investigated the medicinal effect of Food Plant (*Moringa oleifera*). They stated that it has cardiac and circulatory stimulant, antitumor, antipyretic, antiepileptic, antiinflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities. They are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

Mantawy et al (2012): conducted a study concerning the Antioxidant and schistosomicidal effect of *Allium sativum* and *Allium cepa* against *Schistosoma mansoni* different stages. They tested there in vitro effects against miracidiae, schistosomulae, cercariae and adult worms. Their Results indicate a strong biocidal effect against all stages of the parasite and also showed scavenging inhibitory effect on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and nitric oxide (NO).

Recently Farouk et al (2015): Studied the antiviral, antimicrobial and schistosomicidal activities of Eucalyptus camaldulensis Essential Oils. They found that these oils are active candidates and could be used as RNA antiviral, antimicrobial and schistosomicidal agents in new drugs preparation for therapy of infectious diseases.

Table 1 Worm load and percentage reduction in control untreated and treated animals (Immediately after and 10 minutes later)

Animal group	worm load				% total worm reduction
	male worm	female worm	couple worm	total worm	
Subgroup I Infected control untreated	6.5± 2.12	2.5± 1.8	7.0± 2.41	23± 2.56	
Subgroup II Infected mice wiped with 100 µl/mouse Camphor oil 10 min later with 80 <i>S. mansoni</i> cercariae	0***	0***	0***	0***	100***
Subgroup III Infected mice wiped with 100 µl/mouse Camphor oil then infected immediately after with 80 <i>S. mansoni</i> cercariae	0***	0***	0***	0***	100***

Table 2 Tissue egg load and oogram pattern in control untreated and treated animals (Immediately after and 10 minutes later)

Animal group	Mean total percentage of ova ± SD			Mean Number of ova/ gm tissue			
	immature ova	mature ova	dead ova	Liver	% reduction	Intestine	% reduction
Subgroup I Infected control untreated	52.6± 2.4	41.8± 1.92	5.6± 1.52	2896± 603		1827± 477	
Subgroup II Infected mice wiped with 100 µl/mouse Camphor oil 10 min later with 80 <i>S. mansoni</i> cercariae	0***	0***	0***	0***	0***	0***	100***
Subgroup III Infected mice wiped with 100 µl/mouse Camphor oil then infected immediately after with 80 <i>S. mansoni</i> cercariae	0***	0***	0***	0***	0***	0***	100***

Last but not the least; Camphor oil can be effective to relieve minor respiratory issues, such as coughing, sore throat and nasal congestion. Some products can be purchased that can be rubbed into the chest area, which causes that area to warm and some pain can be alleviated.

<http://www.solsticemed.com/Articles.asp?ID=286>.

Further studies to test the components extracted from Camphor oil leaves by chromatography or spectroscopic methods should be taken into consideration in the future.

Table 3 Granuloma measurement and its' percentage reduction in control untreated and treated animals (Immediately after and 10 minutes later)

Animal group	number of granuloma in 10 successive power fields (10x10)	% reduction in number of granuloma	mean granuloma diameter in μm	% reduction of mean granuloma diameter
Subgroup I Infected control untreated	10.43 \pm 2.87		348.54 \pm 11.83	
Subgroup II Infected mice wiped with 100 μl /mouse Camphor oil 10 min later with 80 <i>S. mansoni</i> cercariae	0***	100***	0***	100***
Subgroup III Infected mice wiped with 100 μl /mouse Camphor oil then infected immediately after with 80 <i>S. mansoni</i> cercariae	0***	100***	0***	100***

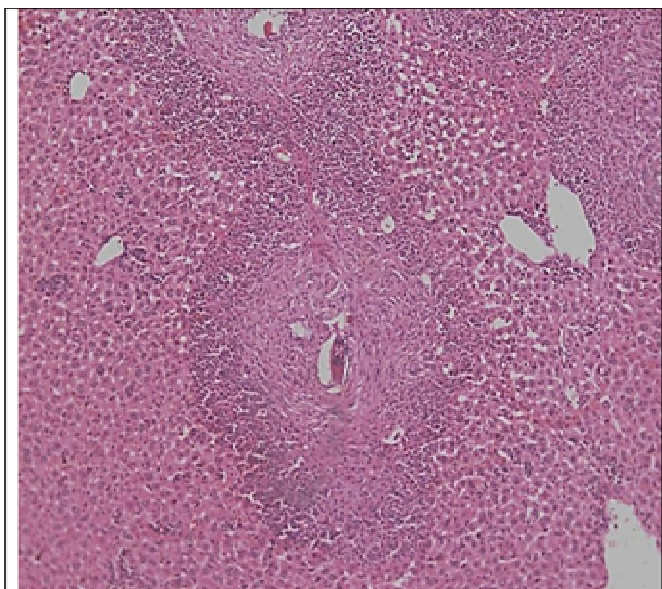


FIG 1. LIVER SECTION FROM INFECTED 80 SCHISTOSOMA MANSONI CERCARIAE UNTREATED CONTROL MOUSE SHOWING LARGE GRANULOMA FORMED OF CENTRAL LIVING MIRACIDIUM SURROUNDED BY LARGE NUMBER OF LYMPHOCYTES,

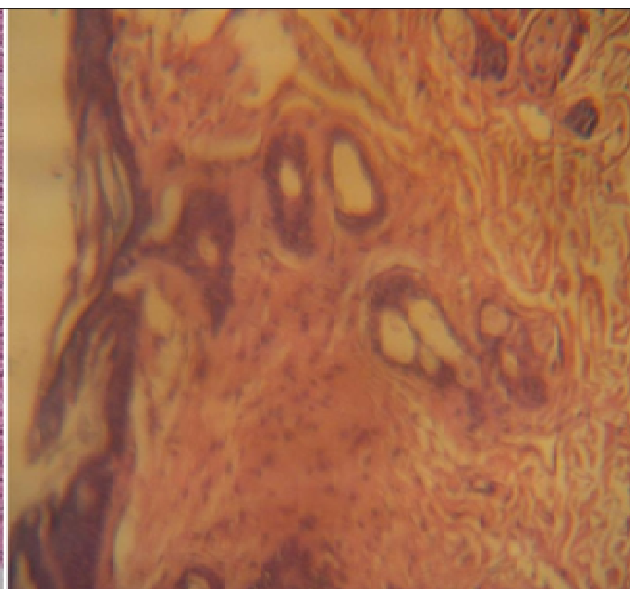


FIG.2. PATHOLOGICAL SKIN SECTION IN EPI- DERMIS OF CONTROL INFECTED UNTREATED MICE AFTER THEIR EXPOSURE TO 80 *S. MANSONI* CERCARIAE/MOUSE SHOWING PENETRATING CERCARIAE AND INFLAMMATORY CELLS (H&E X 200).

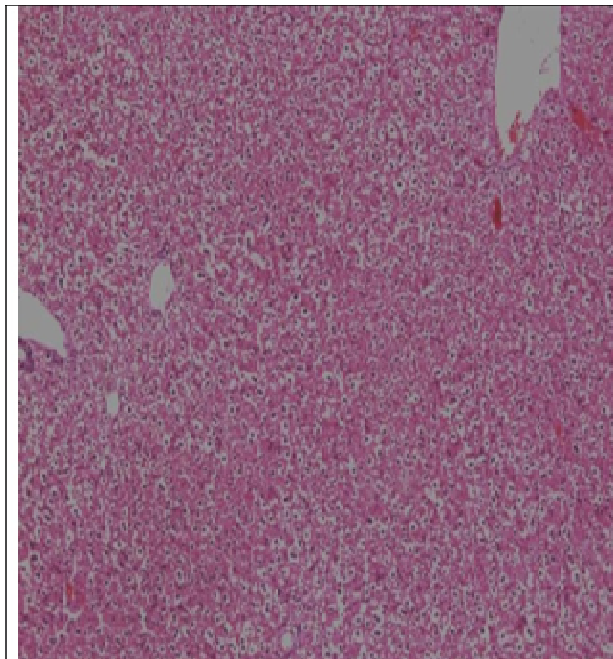


FIG 3. LIVER SECTION FROM INFECTED CAMPHOR OIL ADMINISTERED MICE 10 MINUTES BEFORE INFECTION WITH 80 SCHISTOSOMA MANSONI CERCARIAE SHOWING HEALTHY HEPATIC PARENCHYMA (H & E X 200)

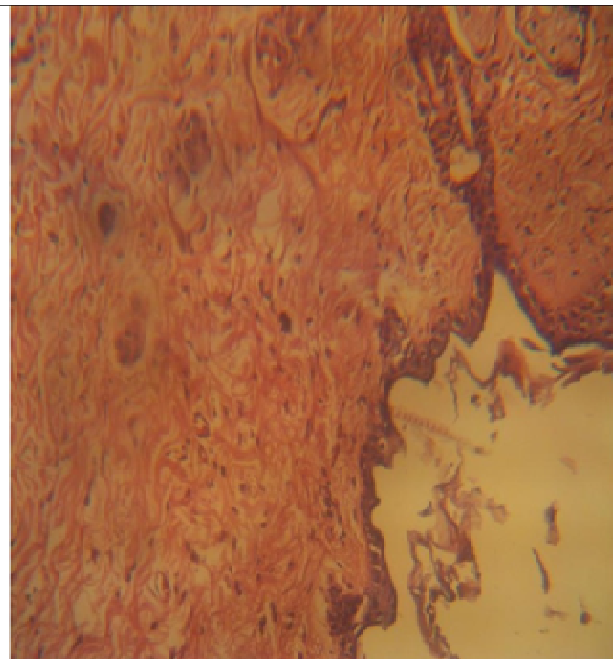


FIG 4. PATHOLOGICAL SKIN SECTION IN EPIDERMIS OF INFECTED CAMPHOR OIL ADMINISTERED MICE 10 MINUTES BEFORE INFECTION WITH 80 SCHISTOSOMA MANSONI CERCARIAE SHOWING STANDSTILL CERCARIAE UNDERNEATH THE EPIDERMIS (H&E X 200).

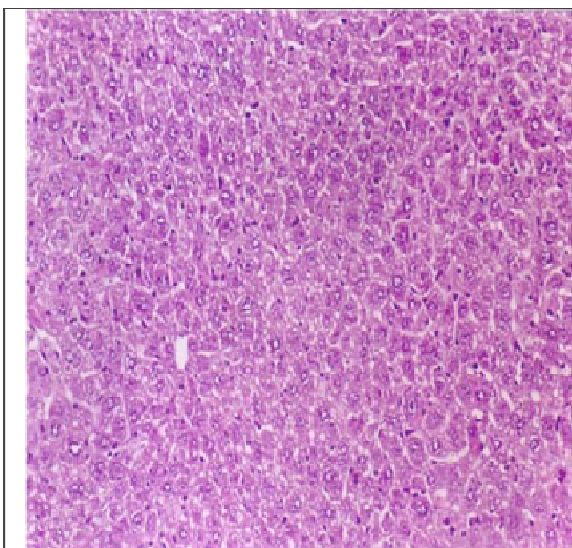


FIG. 5. LIVER SECTION FROM INFECTED, CAMPHOR OIL ADMINISTERED MICE IMMEDIATELY BEFORE INFECTION WITH 80 SCHISTOSOMA MANSONI CERCARIAE SHOWING HEALTHY HEPATIC PARENCHYMA (H & E X 200)

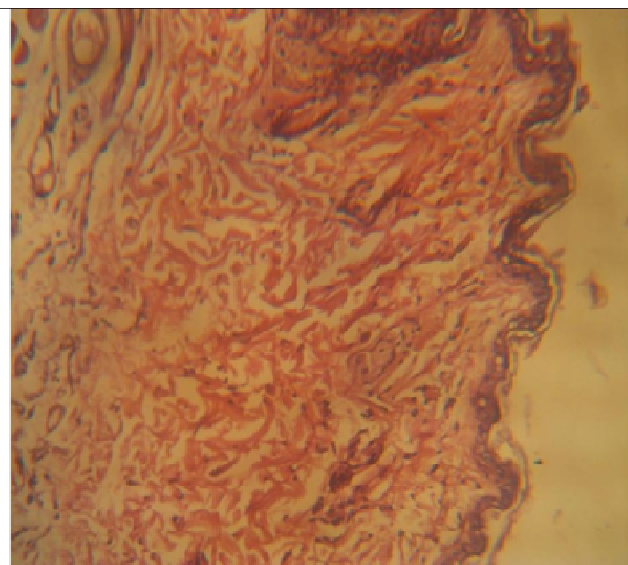


FIG 6. PATHOLOGICAL SKIN SECTION IN EPIDERMIS OF CAMPHOR OIL ADMINISTERED MICE IMMEDIATELY BEFORE INFECTION WITH 80 SCHISTOSOMA MANSONI CERCARIAE SHOWING COMPLETE HINDERING OF CERCARIAE PENETRATION (H&E X 200).

CONCLUSION

This study has been initiated to clarify the prophylactic imprint of topical administration of Camphor oil in experimental schistosomiasis mansoni infection. It could be of utmost help in Egypt, where indigenous people and foreign tourists could wipe this compound locally immediately before fishing or rowing in the Nile River.

RECOMMENDATIONS

Further trials are being recommended to discover new cercaricidal compounds which could be wiped locally on the skin with maximal hindrance to cercarial penetration, and minimal lethal effect to the host skin. This experiment complies with the current laws of the country in which they were performed, which is Egypt.

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