

HISTOPATHOLOGICAL EVALUATION OF A NEW SCHISTOSOMICIDAL DRUG FROM MYRRH

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ABSTRACT

We reported early the biochemical and toxicological results of a new schistosomicidal drug from myrrh (*Commiphora molmol*) (Badria et al.2001). In this study, a histological evaluation of myrrh preparation at therapeutic doses (250 and 500 mg/kg) on Swiss mice infected for 45 days with *Schistosoma mansoni* cercaria was examined. Liver sections were stained with H and E, and Masson's trichome for detection of collagen fibers; Gordon and Sweet for reticular fibers. The results revealed a significant decrease in the number and size of granuloma in mice treated with myrrh preparation at 500 mg/kg. Also, at this dose there was a marked decrease in the intensity of collagen and reticular fibers and decrease of liver fibrosis. These results proved that myrrh preparation has both antifibrotic and schistosomicidal activity which may be explained as the excessive deposition of collagen in several types of lesions is known to undergo resorption when provocative causes are removed.

KEYWORDS: Histology, collagen fibers, reticular fibers, granuloma, myrrh.

INTRODUCTION

In most developing countries, the use of indigenous, natural drugs is a common practice because life-saving synthetic drugs are beyond the reach of the people. In countries such as Egypt, China, and India, it is not only the unavailability or inaccessibility of modern pharmaceuticals that drives people to traditional remedies but, more importantly, the presence of a medical system enshrined within their customs. These herbal remedies are used in the treatment of a wide variety of infectious diseases, including schistosomiasis, which is regarded as a silent disease. Schistosomiasis is a debilitating, and sometimes deadly, parasitic infection that afflicts hundreds of millions of people in developing countries (Taylor *et al.*, 1987; El-Alamy & Cline, 1977). Great efforts have been devoted to develop safe and effective schistosomicidal agents. Other drugs have been developed; however, they were not free from limiting drawbacks. The development of praziquantel is considered a hallmark on the path to eradication of schistosomal infection (Pearson & Guerrant, 1983; Andrews *et al.*, 1983). The use of the drug has been correlated with some side effects such as hyperglycemia and, thus, the search for a new effective and safe schistosomicidal agent is highly encouraged (El-Hawey *et al.*, 1990; Lian *et al.*, 1991; Asfaw *et al.*, 1988). Myrrh (Arabian or Somali Myrrh)

is an oleo-gum resin obtained from the stem of *Commiphora molmol*, Engier, and probably other species, of the Burseraceae (Greene, 1993). Myrrh contains a resin (myrrhin), 23–40%; a volatile oil (myrrhol), 2–8%; gum, 40–60%; and a bitter principle (Al-Awadi *et al.*, 1991; Claeson *et al.*, 1991). The volatile oil contains terpenes sesquiterpenes (furanogermacranes, furanoguaianes and furanoeudesmanes), esters, cuminic aldehyde and eugenol (Moran *et al.*, 1992; Michiel & Cooper, 1991; Chevallier, 1996). The chemistry of the resin is not fully elucidated. It is generally classified to ether-insoluble (smaller) portion which contains harrabomyrrholic acids and on ether-soluble (larger) portion which contains com-miphoric acids, the ester of another resin acid, commiphorinic acid and two phenolic resins, and harrabomyrrhol (Trease & Evans, 1989). Myrrh is useful for treatment sore throat, bleeding gums, chronic pharyngitis, and amenorrhia (Claeson *et al.*, 1991; Moran *et al.*, 1992). Myrrh is used widely in Somalia for the treatment of diarrhea and stomach complains (Michiel *et al.*, 1991). Moreover, myrrh is approved by the Food and Drug Administration (FDA) for food use (21 CFR 172.510) and was given status as a flavor ingredient (No.2765) by FEMA. The council of Europe (1981) included myrrh in the list of plants and parts thereof, which are acceptable for use in foods. Recently, Badria *et al.* (2001) reported that oral administration

of myrrh preparation resulted in a significant reduction in the of worm burden of mice. It also induced separation of male-female coupled worms. Moreover, it shifted female worms from their normal habitat to the liver. Coincident with the shift of the female worms to the liver, a progressive reduction in the number of immature eggs laid in the wall of the small intestine was proved. This effect was accompanied by an increase in the percentage of the mature stage from 14.9 ± 1.7 in control animals to 93 ± 7.5 and 93.4 ± 8.2 in animal treated with myrrh preparation at doses 250 and 500 mg/kg, respectively. Therefore a histological evaluation of myrrh preparation at therapeutic doses (250 and 500 mg/kg) on Swiss mice infected for 45 days with *Schistosoma mansoni* cercaria was necessary to assure the safety, efficacy, and its role as antifibrotic agent.

MATERIAL AND METHODS

One kilogram of powdered myrrh (*Commiphora molmol*) was extracted with petroleum ether 40–60°C (3 X 1.0 L) by percolation at room temperature. The combined extracts were evaporated under vacuum at 40 °C to give (fraction A) as a pale yellow viscous liquid. The defatted powder was extracted with methanol (3 X 1.0 L) by percolation at room temperature. The combined extracts were evaporated under vacuum at 40 °C to dryness. The obtained yellowish brown residue was dried and powdered to give fraction B as a yellow fine powder. Fractions A and B were mixed in calculated amounts to give the finished product (myrrh preparation).

Laboratory infection of experimental animals

A cercarial suspension obtained from infected snails (Theodor Bilharz institute, Cairo, Egypt) was mixed and 0.2 ml was transferred using a 1 ml syringe to a staining petridish cover. Few drops of buffered formalin and iodine solution were added to kill and stain cercariae, respectively. Cercariae were counted under dissecting microscope and the cercarial suspension was adjusted so that each 0.2 ml of the suspension contains approximately 60 cercariae. Male albino mice (local bred) were infected subcutaneously with 0.2 ml of the suspension and kept *ad libitum* for 45 days to ensure complete maturation of worm and excretion of eggs in the feces. Forty-five days after infection with schistosomal cercariae, mice were placed in 4 groups of 8 mice each. From each group, two mice were randomly selected and checked for the presence of mature schistosomes in their livers and mesenteric blood vessels. The presence of mature viable eggs in their stools was also examined.

Calculation of granuloma number and size:

In liver sections stained with H & E, the hepatic granuloma number in ten sections from each mouse was calculated in a defined area (0.5 cm) using an ocular micrometer. Also, the mean size of ten granuloma per section were measured (i.e. 100 granulomas/mouse) using ocular micrometer (9).

The schistosomicidal effect was accompanied by an increase in the percentage of the mature stage form 14.9 ± 1.7 in control animals

Table 1 : The mean granuloma number and size among infected, non-treated and treated groups

Animal group	Mean granuloma number (\pm SEM)	Mean Granuloma size, μm^2 (\pm SEM)
Non-infected	-	-
Infected and Non-Treated	13.2 ± 1.2	150 ± 6.3
Infected and treated with praziquantel (250mg/kg)	$1.8 \pm 0.3^*$	$30 \pm 3.8^*$
Infected and treated with myrrh (250 mg/kg)	$2.8 \pm 0.5^*$	$40 \pm 5.0^*$
Infected and treated with myrrh (500 mg/kg)	$1.9 \pm 0.6^*$	$33 \pm 2.9^*$

* Significantly different from the infected non-treated mice ($p < 0.001$).

to 93 ± 7.5 and 93.4 ± 8.2 in myrrh-treated animals, 250 and 500 mg/kg, respectively (Table 2). Administration of praziquantel produced similar responses.

Statistical analysis

Data are presented as mean \pm SEM. The difference between means was assessed using Student's t-test. A p value less than 0.05 was considered to be statistically significant.

Results

Sections of liver stained with H and E showed granulomas formed of eggs and concentrically arranged mononuclear leucocytes and fibroblast. In mice infected with myrrh preparation at dose 250 and 500 mg/kg, the number and size of granulomas were decreased. The granuloma number and size were significantly reduced at 500 mg/kg ($p < 0.01$) and insignificantly reduced at 250 mg/kg (Table 1). The liver sections stained with Masson's trichrome collagen fibers stained blue and appeared in the capsule, interstitial spaces, the wall of central vein and portal vein. In control mice, the amount of collagen fibers is low. In infected mice there was a marked increase of collagen fibers. Also collagen fibers formed lamellar fibrosis around the trapped eggs. Collagen was less dense after treating the mice with 250 mg/kg of myrrh preparation. The granuloma size and number were reduced because of the reduction periovular collagen fibers. The distribution of the collagen fibers in infected mice after treatment with 500 mg/kg myrrh preparation showed almost normal pattern of normal non-infected mice.

Discussion

One of the most accessible health care providers for most developing countries is the traditional medical practitioner who has in his possession an armamentarium of effective herbal

remedies. Some of these herbal remedies are used for treatment of a wide variety of infectious diseases, including schistosomiasis which is regarded as a silent disease in many countries. Myrrh is one of the oldest known medicines and was widely used by ancient Egyptians. Liver sections stained with Gordon and Sweet for reticular fibers, these fibers appeared as black fibers. The distribution of reticular fibers in normal appeared in the capsule, interstitial space and the walls of central veins. In infected mice, there were marked increase of reticular fibers in periovular granulomas. The majority of granulomas were formed of both reticular and collagen fibers and some of them were formed of reticular fibers only. In mice treated with myrrh preparation at 500 mg/kg, there was a marked decrease of reticular fibers in the granulomas.

A post-infection treatment with myrrh 250 and 500 mg/kg significantly reduced those measures in a manner that correlated well with the observed schistosomicidal activity. As compared to the marketed schistosomicidal drug, Praziquantel™, the rank order of potency in protecting against liver cell damage was praziquantel > myrrh preparation 500 mg/kg > myrrh preparation 250 mg/kg; a relative potency that is congruent with the schistosomicidal activity of these drugs. This implies that 500 mg/kg might be a better dosage regiment than the 250 mg/kg. The wide safety margin (low toxicity) found for myrrh preparation may augment this concept. The low toxicity of myrrh preparation, relative to praziquantel, is a further merit and promise in favor of this newly emerging drug. However, the nature of myrrh preparation as an oleogum resin leads to speculation about possible direct hepatoprotective effects. These results encourage the continuation of further experimental and clinical study to develop such a unique, safe, and effective antischistosomal drug of natural origin.

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