In vitro Efficacy of a Combination of Stromectol and Nigella sativa Oil Against Cestodes in Sheep, Taif, Saudi Arabia

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Abstract: The current study had been carried out in vitro to investigate the comparative morphological effects of Stromectol/Nigella sativa oil combination and each of them separately against adult Moniezia expansa. The live adult parasites were collected from naturally infected sheep slaughtered in Taif abattoir, then exposed to 50 ng/ml Stromectol, 1 mg/ml N. sativa oil extract and a combination of Stromectol and N. sativa oil in half concentration. Surface changes to the parasites were assessed by scanning electron microscopy. The results revealed that the combination of half dose of Stromectol/N. sativa oil was more destructive to the tegumental surfaces of adult cestodes than each of them separately. The present study had provided morphological evidence for the greater anthelmintic activity of ivermectin Stromectol on combination with N. sativa oil and revealed that combination had been shown to be synergistic against Moniezia expansa infections in sheep.

Key words: Moniezia expansa • Stromectol • Nigella sativa Oil • Combination Therapy • In vitro

INTRODUCTION

Sheep production is an attractive source of income for farmers in Saudi Arabia. However, productivity is constrained by parasitic infections. Helminth infections are amongst the most common parasitic infections of animals worldwide and are now well recognized as an important veterinary problem, both in developing and in developed countries [1, 2].

The sheep industry still relies heavily on the use of anthelmintics to alleviate the infections of gastrointestinal nematodes, cestodes and liver flukes that are the most important. At a time, anthelmintic resistance has become a serious problem in veterinary medicine [3, 4]. A number of strategies have been proposed to try and preserve the efficacy of existing drugs and slow down the spread of resistance. Strategies involve the use of alternative anthelmintics, the rotation of drugs from different treatment chemical groups, the selective treatment of animals and the use of drug combinations [5]. Combinations of two or more drugs are a routine part of parasite control in the livestock. They can be used to treat mixed infections as triclabendazole (the fluke drug) is used in combinations with drugs target nematodes, such as levamisole, oxfendazole, ivermectin, or abamectin. Using anthelmintics from different chemical groupings (and with different mechanisms of action) achieve an additive or synergistic effect. With synergistic combinations, the possibility of using lower-than-normal concentrations would reduce drug costs and side-effects and reduce drug residues in host tissues and in the environment. A combination of mebendazole and levamisole has been shown to be synergistic against Haemonchus contortus in sheep [6]. Febantel and pyrantel showed synergistic action against Ancylostoma caninum in dogs [7] and Heterakis spumosa in mice [8]. Moreover, febendazole and pyrantel proved synergistic effect against Toxocara canis in vitro [9]. The present study reports on the activity of an ivermectin/Nigella sativa oil combination to enable treatment of both gastrointestinal helminthes and trematode infections simultaneously, since ivermectin is a very effective anthelmintic for the treatment of nematode infections [10, 11] and Nigella sativa oil has high activity against cestodes and liver flukes [12, 13]. Present study was carried out in vitro to examine the morphological effects of a(stromectol) ivermectin/ Nigella sativa oil combination against adult cestode (Moniezia expansa).

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MATERIALS AND METHODS

Drug: Stromectol (Ivermectin) is a semisynthetic anthelmintic agent against a wide variety of nematode parasites.

Stromectol: Active Ingredient of Ivermectin

Other Names for this Medication: Ivectin, Ivenox, Ivera, Ivergot, Ivermec, Ivermectina, Ivermectine, Ivermectinum, Ivert, Ivexterm, Kilox, Mectizan, Quanox, Revectina, Scabo, Securo, Simpiox.

Preparation of N. sativa Extract: The dried seeds of N. sativa were purchased from a local market at Taif, Saudi Arabia. The seeds were crushed and cold macerated in petroleum ether (40-60°C) for three days. After evaporation of petroleum ether, the extract was taken out and the oil was filtered. The extracted oil was kept in screw-capped tubes in the dark at -20°C until use [14].

Anthelmintic Effects of Stromectoland/or N. sativa Oil:

Adult worms of Moniezia expansa were collected from intestine of naturally infected sheep slaughtered in Taif abattoir and washed in several changes of warm (37°C), sterile complete RPMI 1640 culture medium containing antibiotics (penicillin, 50 IU/ml; streptomycin, 50 mg/ml). The worms were subsequently transferred to fresh culture medium containing 50 ng/ml stromectol; corresponded to maximum blood levels in vivo [15] or N. sativa oil extract at concentration of 1 mg/ml; the lowest concentration that could kill adult worms in vitro or both stromectol and N. sativa oil in half concentration. The N. sativa oil was initially prepared as a stock solution in DMSO and added to the culture medium to give a final solvent concentration of 0.03%(v/v). Other group was prepared by incubating worms in RPMI 1640 culture medium containing 0.03%(v/v) DMSO; as solvent control. The worms were incubated for 24 h at 37°C in an atmosphere of 5% CO2. Normal control worms at 0 h were fixed immediately following the initial washing. Six worms were examined for each group.

Scanning Electron Microscopy (SEM): Following incubation, the anterior end of adult worms was fixed intact for 12 h in a 3:1 mixture of 4% (w/v) glutaraldehyde, 0.12 M-Millonig’s buffer, pH 7.4 and 1% aqueous osmium tetroxide. Then specimens were processed for SEM following a method previously reported [16].

RESULTS

SEM of Normal Fresh Worms: The scolex appeared globular and was provided with four oval suckers radially located around the proximal end of the scolex (Fig. 1a).

The strobila was an elongated ribbon-like structure composed of series of segments (proglottids) which were broader than longer (Fig. 1b). Each segment contained a common genital pore on either side (Fig. 1b inset).

SEM of Treated Worms: After 24 h incubation with 50 ng/ml stromectol, the changes in adult cestodes concerned the proglottides other than the scolex which retained its normal morphology (Fig. 1c). The proglottides lost their normal aspect showing wrinkled tegumental surface throughout the strobila (Fig. 1d). After 24 h incubation with 1 mg/ml N. sativa oil, scolex appeared to be more swollen than normal with blebs of varying sizes covering the surface with contractions to the sucker’s opening (Fig. 1e). The proglottides appeared deformed with wrinkled surface (Fig. 1f and inset).

The changes in adult cestodes after 24 h incubation with drug combination (25 ng/ml stromectol and 0.5 mg/ml N. sativa oil) concerned the whole-body surface. While the surface of the suckers appeared slightly swollen (Fig. 1g), the general tegument appeared distorted. There was a difference in the degree of disruption in tegumental surface between the examined specimens. In some specimens, severely folded and corrugated tegument was observed (Fig. 1h). In other specimens, tegumental swelling occurred so that the proglottides could not be distinguished (Fig. 1i).

DISCUSSION

The present study demonstrated the comparative morphological effects of stromectol/N. sativa oil combination and each drug separately against cestode parasites, Moniezia expansa.

The SEM observations showed that the combination of half dose of stromectol/N. sativa oil was more destructive to the tegumental surfaces of adult helminthes than either drug separately. The surface changes observed in M. expansa after treatment with the drug combination were more severe than those after treatment with stromectol alone, in that tegumental distortion was more widespread, extending into the scolex.
Fig. 1: Scanning electron micrographs (SEMs) of the anterior end of adult *Moniezia expansa*. (a, b) SEMs of normal fresh worm. SC, scolex, S, suckers, ST, strobila (c, d) Changes after 24 h of incubation in 50 ng/ml stromectol. (e, f) Changes after 24 h of incubation in 1 mg/ml *N. sativa* oil. (g, h, i) Changes after 24 h of incubation in a combination of half-strength stromectol and half-strength *N. sativa* oil
of the cestode and the lateral margins of the parasites. While, the general level of tegumental disruption in *M. expansa* was affected by using both *N. sativa* oil and drug combination-treated groups.

Indeed, ivermectin had a potent antinematodal shown to be activity in a variety of domestic animals, but it considered to be insusceptible versus platyhelminths [17]. The anthelmintic activities of *N. sativa* oil were studied by Agarwal *et al.* [18] who reported that the essential oil from the seeds of *N. sativa* showed pronounced activity even in 1:100 dilutions against tapeworms and earthworms. Anticestodal effects of *N. sativa* seeds were studied in children infected naturally with the respective worms.

A single oral administration of 40 mg/kg of *N. sativa* oil extract reduced the percentage of the fecal egg without producing any adverse side effects [19]. When given orally to *Schistosomamansoni*-infected mice, 2-week treatment with *N. sativa* oil reduced the number of *S. mansoni* worms in the liver and decreased the total of number of ova deposited in both the liver and the each drug intestine [20]. Furthermore, it increased the number of dead ova in the intestinal wall and reduced the granulomadiameters markedly. When *N. sativa* oil was administered in combination with praziquantel, the drug of choice for the treatment of schistosomiasis, the most prominent morphological changes induced by the particular effect was a further lowering of the dead ova number over that produced by praziquantel alone [21].

Passive diffusion of morphological evidence for the greater anthelmintics through the cuticle [22] would probably be responsible for destructive changes and deformation of the nematode body surface [23, 24]. Also, the general body surface of platyhelminth parasites acts as an absorptive surface. In the present study, the helminth’s body surface was observed to be affected and altered by *N. sativa* oil and drug-combination. Similar to the present observations, the surface cuticle or tegument was found to be a principal target site for different synthetic drugs and natural anthelmintic products as proved by veterinary histomorphological and ultrastructural studies [25-22]. The tegument of cestodes and trematodes were known to be the basic entry route and primary site of activity of anthelmintic drugs [18, 23].

*N. sativa* seeds contained fixed oils and volatile oils, which were rich sources of quinones, unsaturated fatty acids, amino acids and proteins in addition to traces of alkaloids and terpenoids. Most of the studies on the biological effects of *N. sativa* had dealt with its crude extracts in different solvents; however, some studies used its active principles. Among the components isolated from the volatile oil of *N. sativa*, thymoquinone had been shown to be the principal active ingredient [24]. *In vitro* physicochemical assays characterized that component as cytotoxic. This active principle in *N. sativa* oil had been found to exert cytotoxic effects both *in vitro* and *in vivo* against various tumor cells [25]. Therefore, it was reasonable to suggest that the greater disruption to the surface morphology of the combination-treated parasites might be attributed to an additive or synergistic effect between ivermectin and *N. sativa* oil with different modes of action. Few SEM studies had been carried out to determine the synergistic potential of drug combinations.

**REFERENCES**


