

## Conjoined Efficacy of Antioxidants with Anthelmintics in Treatment of Helminthic Infection in Mice

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**Abstract:** “Back to nature” is an increased demand nowadays, for using plants in therapy “instead of using synthetic drugs that may have adverse effects. Grape seed extract (Proanthocyanidins) and Selenium possesses a wide array of pharmacological and biochemical actions. *Hymenolepiasis* and *Passalurus* infections are considered as common health problems worldwide. In the present study, Grape seed extract and Selenium were evaluated singly or in combination with Anthelmintics (Niclosamide or Mebendazole) in treatment of *Hymenolepis nana*, *Hymenolepis diminuta* and *Passalurus ambiguous* infections in 160 naturally infected mice. Treatment of *H. nana* & *H. diminuta* infected mice with Niclosamide (half therapeutic dose) gave an efficacy of 47% while on addition of Grape seed extract and Selenium the efficacy of the drug increased to 64%, 57% respectively ( $P \leq 0.05$ ). In case of *Passalurus ambiguous* infected group Mebendazole (half therapeutic dose) gave an efficacy of 50% ( $P \leq 0.05$ ). With addition of Grape seed extract and Selenium the efficacy of the drug was 55.3% for both. Complete disappearance of eggs in feces was obtained using full therapeutic dose of the specific drug alone while treatment using antioxidants alone revealed an efficacy ranging from (12.4-24.7%) in the two infected groups without significance ( $P \leq 0.05$ ). It is concluded that the use of medicinal plants hold hope of finding new Anthelmintics treatment as part of an integrated approach specifically designed to achieve sustainable parasite control.

**Key words:** Antioxidants, Anthelmintics, helminthic infection, mice.

### INTRODUCTION

Grape seed extract (Proanthocyanidins) is considered to have anti-inflammatory and anti-carcinogenic activities (Bagchi *et al.*, 2000). Also it is considered to be a potent antioxidant and free radical scavenger, chemoprotective and a cytoprotective agent. Selenium, which is a trace element, can modulate the immune function and influence the susceptibility of the host to infection. It has an influence on macrophage function (Rivera *et al.*, 2002). Moreover, Selenium has antioxidant properties (Chu *et al.*, 2004).

*Hymenolepiasis* and *Passalurus* infections are common health problems worldwide (Cabello, 2007). This tapeworm is endemic in Asia, Africa, and southern and eastern Europe. Its life cycle involves humans or rodents as the definitive host and arthropods as the intermediate host (Cho *et al.*, 2009). *Passalurus ambiguous* is an intestinal nematode belonging to the family Oxyuridae. Its inhabitant is the caecum and colon of mice, rats and hamsters with variable prevalence (Huggot, 1984). Niclosamide (Yomesan) is an effective broad spectrum anti-cestodal drug (Marangi *et al.*, 2003). Mebendazole (Antiver) is also a broad spectrum anthelmintic. It possesses vermifugal and ovicidal activities with a high cure rate against most gastrointestinal tract helminths (Cañete *et al.*, 2009).

In the present study, Grape seed extract and Selenium were evaluated singly or in combination with Niclosamide or Mebendazole in treatment of *H. nana*, *H. diminuta* and *P. ambiguous* infections in naturally infected mice.

### MATERIALS AND METHODS

#### Drugs:

- Niclosamide (Yomesan) Alexandria Co. for Pharmaceuticals. Each tablet contains 0.5 g (the market form of the drug).
- Mebendazole (Antiver) Alexandria Co. for Pharmaceuticals. A 30 ml suspension. Each 5 ml contains 100mg of Mebendazole.
- Selenium: Each tablet contains (Selenium yeast 1000 $\mu$ /g, Vit A acetate 500 IU/mg, Ascorbic acid 97% and Vit E acetate 15 mg). Arab Co. for Pharm. & Medicinal Plants MEPACO.
- Grape seed extract, ethanol extraction using Chromatography (Personal Communication).

**Drugs Doses:**

Mice doses of the tested drugs Niclosamide (Yomesan) and Mebendazole (Antiver) were calculated by multiplying the total human dose in 0.0026 (mice factor) according to Paget and Barnes (1964). The calculated dose was administered as a water suspension at the root of the tongue of the mice by the aid of a special syringe (plastic syringe supported by a suitable needle with rounded tip). Drugs and antioxidants were administered as recommended by the producer company as follows:

Mebendazole, was given as three successive doses at one week interval. While Niclosamide was given as a first initial dose (5.2 mg/mice) followed by six daily doses of (2.6 mg/mice). Mice were examined one week post the last administered dose.

One hundred and sixty, naturally infected albino male and female mice weighing (20-22gm) were selected from the animal house of The Research Institute of Ophthalmology. Individual animal stool was examined parasitologically for calculation of the mean eggs shed/g feces using direct smear, concentration flotation methods and *McMaster* egg-counting technique which was used for detection of *Passalurus* eggs (Weber *et al.*, 1992). Stool examination was performed for each mice pre and post treatment to identify the present infection and the effect of treatment. Examination was performed in the animal house of Faculty of Veterinary Medicine, Cairo University. Mice selected for the study were harboring single infection of *H. nana*, *H. diminuta* or *P. ambiguous*. Mice were housed in polypropylene cages at 25±2°C. Mice were fed dry ration and water at libidum. The animals were divided into two main infected groups: *Hymenolepis* and *Passulurus* infected group. Each type of infection was divided into eight groups, 10 each, as designed in (Tables 1 & 2). The protocol was approved by the Animal Ethics Committee of the Faculty of Medicine, (Ethical Clearance No. AEKKU48/2552).

**Table 1:** Experimental Design of Administered Doses & Combinations in the *Hymenolepiasis* infected group.

Group of Mice	Number of the Group	Treatment Regimen	Dose of the Tested Drug
<i>H. nana</i> , <i>H. diminuta</i> Infected	Group I	Niclosamide	5.2 mg/mice
	Group II	Niclosamide	2.6 mg/mice
	Group III	Niclosamide + Antiox	2.6mg/mice + 260microgram/mice
	Group IV	Niclosamide + Grape Seed Extract	2.6mg/mice + 260microgram/mice
	Group V	Antiox	260microgram/mice
	Group VI	Grape Seed Extract	260microgram/mice
Infected Not-Treated	GroupVII	-	-
Non Infected Control	GroupVIII	Water Only	-

**Table 2:** Experimental Design of Administered Doses & combinations in the *P. ambiguus* Infected group.

Group of Mice	Number of the Group	Treatment Regimen	Dose of the Tested Drug
<i>P. ambiguous</i> Infected	Group I	Mebendazole	5.2 mg/mice
	Group II	Mebendazole	2.6 mg/mice
	Group III	Mebendazole + Antiox	2.6mg/mice + 260microgram/mice
	Group IV	Mebendazole + Grape Seed Extract	2.6mg/mice + 260microgram/mice
	Group V	Antiox	260microgram/mice
	Group VI	Grape Seed Extract	260microgram/mice
Infected Not-Treated	Group VII	-	-
Non Infected Control	Group VIII	Water Only	-

Efficacy of the drug was calculated depending on the difference in the mean number of shed eggs/g feces between the treated as well as with the control untreated animals. Also Efficacy was calculated for each mouse between the level of mean eggs shed/g feces before and after treatment. Efficacy was calculated according to the following equation:

$$\text{Efficacy} = \frac{A-B}{A} \times 100.$$

A= No. of eggs extracted from control animals.

B= No. of eggs extracted from treated animals.

**Statistical Analysis:**

Data were statistically described in terms of mean ± standard deviation (± SD), and compared using Wilcoxon signed rank test for paired (matched) samples. Values were considered statistically significant when  $P < 0.05$ . All statistical calculations were done using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

**RESULTS AND DISCUSSION**

Concerning efficacy of the tested drugs and their combinations on *Hymenolepis* naturally infected mice groups, Data in Table (3) revealed that oral administration of Niclosamide in its therapeutic dose caused

disappearance of *Hymenolepis* species eggs shed/g feces in (Group I) of infected mice. Efficacy was 100%, with significant difference ( $P < 0.05$ ) in comparison with the number of eggs shed/g feces of the same mice group before treatment as well as with the control untreated mice group. The efficacy of Niclosamide decreased to 47% on using half of the therapeutic dose in treatment of Group II infected mice with *Hymenolepis* species without significant difference. In the present study two antioxidants were evaluated. Combination of Grape seed extract with Niclosamide (half therapeutic dose) in Group IV gave an efficacy of 64% with significant difference ( $P < 0.05$ ) in comparison with the number of eggs shed/g feces of the same mice group before treatment as well as with the control untreated mice group. These obtained results are higher than that obtained on using Selenium in combination with the same drug in Group III which gave an efficacy of 57% without significant difference ( $P < 0.05$ ). In the same time treatment of the corresponding groups (Groups V and VI) using antioxidants alone (Selenium and Grape seed extract) revealed an efficacy of (15%, 24.7%) respectively without significance ( $P < 0.05$ ).

Concerning efficacy of the tested drugs and its combinations on *Passulurus* naturally infected mice groups, Data in Table (4) revealed that oral administration of Mebendazole in its therapeutic dose caused disappearance of *Passulurus* species eggs shed/g feces in (Group I) of infected mice. Efficacy was 100%, with significant difference ( $P < 0.05$ ) in comparison with the number of eggs shed/g feces of the same mice group before treatment as well as with the control untreated mice group. The efficacy of Mebendazole decreased to 50% on using half of the therapeutic dose in treatment of Group II infected mice with *Passulurus* species without significant difference.

Treatment of *Passulurus* naturally infected mice groups using Mebendazole (half therapeutic dose) in combination of the two evaluated antioxidants revealed no difference in efficacy (55.3%) (Groups III and IV). There was no significant difference ( $P < 0.05$ ) in comparison with the number of eggs shed/g feces of the same mice group before treatment as well as with the control untreated mice group. At the same time, treatment using both antioxidants alone doesn't reveal any considerable effect with efficacy percentage (12.4%, 22%) for Selenium and Grape seed extract respectively without significance ( $P < 0.05$ ) (Groups V and VI).

**Table 3:** Efficacy of different treatments on *H. immitis* infected mice.

Tested Group	Difference in mean EPG before and after treatment	Efficacy %	Significance at ( $P \leq 0.05$ )
Group I	10± 0.62	100%	Significant
Group II	4.6± 0.24	47%	Not-significant
Group III	5.4±0.42	57%	Not-significant
Group IV	6.4± 0.33	64%	Significant
Group V	2.6± 0.12	15%	Not-significant
Group VI	1.6± 0.1	24.7%	Not-significant
Infected Not- treated Group	0.0	-	-
Non Infected Control	0.0	-	-

**Table 4:** Efficacy of different treatments on *P. ambigus* infected mice.

Tested Group	Difference in mean EPG before and after treatment	Efficacy %	Significance at ( $P \leq 0.05$ )
Group I	300± 10.5	100%	Significant
Group II	133± 3.6	50%	Not-significant
Group III	167± 7.7	55.3%	Not-significant
Group IV	167± 7.7	55.3%	Not-significant
Group V	33± 2.2	12.4%	Not-significant
Group VI	67± 3.3	24%	Not-significant
Infected Not-treated Group	0.0	-	-
Non Infected Control	0.0	-	-

New trends are directed nowadays towards Phyto-medicine to treat parasitism. Many modern commercial medicines are derived from plants. However, scientific evidence on the anti-parasitic efficacy of some plant products is limited. Validation of the anti-parasitic effects and possible side-effects of plant products is necessary prior to their adoption as a novel method for parasite control (Githiori *et al.*, 2006). Helminthic infections of Hymenolepiasis and *Passulurus* are common health problems affecting humans and animals (Cabello, 2007).

From the present study, it is revealed that administration of both Niclosamide and Mebendazole in their full therapeutic dose resulted in complete disappearance of eggs shed/g feces. This can be explained by that Niclosamide causes necrosis of the head and adjoining segments of the worm thus loses its hold and is eliminated either intact or in pieces with the feces (Marangi *et al.*, 2003). Moreover, Cañete *et al.* (2009) reported that Mebendazole blocks glucose uptake by adult worms and larvae. Consequently the energy sources, glycogen and ATP are depleted in the worm leading to their immobilization and death. In the present study, combination of antioxidants with Anthelmintics revealed that Grape seed extract was able to induce synergistic

effect with Niclosamide in treatment of *H. nana* & *H. diminuta* naturally infected mice groups than that produced with Selenium combination with the same drug. However, the use of each antioxidant alone didn't induce any considerable effect.

Synergistic effect of antioxidants with specific antiparasitic drug may occur as a result of the ability of the antioxidant to improve the immune and general health conditions of the treated mice. This could be understood from previous studies which reported that Proanthocyanidins (the active compound in Grape seed extract) possess a wide array of pharmacological and biochemical actions. These included anti-inflammatory, anti-carcinogenic activities and cardioprotective biological effects. Moreover, it helped to reduce allergic responses by inhibiting enzymes involved in the formation of histamine (Zhou *et al.*, 2011). De Souza *et al.* (2002) reported that; in *Trypanosoma cruzi* infected mice; higher susceptibility to infection was found when fed with Selenium-deficient chow. Many authors reported reduced parasitemia and mortality after Selenium supplementation (Davis *et al.*, 1998).

It is worth to mention that Safir *et al.* (2003) recorded that in vitro selenium supplementation enhanced phagocytosis, degranulation by the release of  $\beta$ -glucuronidase after N-formyl-methionyl-leucyl-phenylalanine (FMLP) or cytochalasin B, and the production of superoxide anion after phorbol myristate acetate stimulation of these cells. Selenium supplementation enhanced significantly the release of tumor necrosis factor, interleukin-1 and interleukin-6 compared to selenium-deficient cells.

*Hymenolepis nana* (the dwarf tapeworm) and *Hymenolepis dimmuta* (rat tapeworm) are causative agents of hymenolepiasis which is an infectious disease affecting humans and animals (Barton-Behravesh *et al.*, 2008). *Passalurus ambiguus* is also a nematodal infection that can infect mice (Huggot, 1984). These gastrointestinal infections have adverse and damaging effects leading to the formation of reactive oxygen species (e.g., hydrogen peroxide; hydroxyl radical) and generation of Nitric oxide (Silva *et al.*, 2003). Nitric oxide have damaging effects on various tissues including the gastrointestinal tissues (Boczko *et al.*, 2005). Grape seed extract and Selenium are potent antioxidants and free radical scavengers (Chu *et al.*, 2004). Viveros *et al.*, (2011) reported that Grapes have high amounts of phenolic compounds, which can modulate the gut activity as well as modify the structure and function of the gastrointestinal tract.

In the author's opinion and in agreement with Zhou *et al.*, (2011), addition of Grape seed extract enhanced the immunity. These effects aid in the increase of the efficacy of the tested Anthelmintics due to the dual action in help of these antioxidants. This was on the contrary with that reported by Lluís *et al.* (2011) and Mellen *et al.*, (2010) as they mentioned that grape seed supplementation didn't induce change in biomarkers of inflammation.

Treatment with antioxidants alone led to insignificant level of eradication of infection in the treated mice groups. These data were in agreement with Githiori *et al.*, (2006) and Ketzis *et al.*, (2002) who reported that plant remedies resulted in reductions in the level of parasitism much lower than those observed with antihelminthic drugs or even they have not any reducing effect. The present study revealed higher synergistic effect for antioxidant in treatment of *hymenolepis* infection using Niclosamide than that recorded with Mebendazole in treatment of *Passalurus* infection. This may be related to the difference in feeding nature of cestodal worms in comparison with that of nematodal worms. By the same way, the obtained results of *Passalurus* infected group could be explained. The present study revealed an advantage of antioxidants than the already known ones in treatment program of Anthelmintics as reported by Gupta and Dixit, (2011) in their study for the antioxidant effect of Grape seed extract.

It is concluded from the present study that using antioxidants in combination with specific Anthelmintics improved their efficacy as recorded with the use of half of the therapeutic dose. This opened a new field (after further studies) to use small doses of specific drugs in association with antioxidants to obtain the same results recorded using the full therapeutic dose of these drugs. This increased the safety margin, decreased the development of drug resistance and decreased the drug toxicity caused by the cumulative effect of the drug in the treated animals. Also it is concluded that the use of medicinal plants hold hope of finding new Anthelmintics treatment as part of an integrated approach specifically designed to achieve sustainable parasite control.

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