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# EFFECT OF ANTIOXIDATIVE PROPERTIES OF HONEY ON *SCHISTOSOMA MANSONI*-INFECTED MICE

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Twenty Swiss albino mice were divided into four groups. The first and second group were subjected to intra-peritoneal injection with a honey *Nigella sativa L.* and a honey mixture of *Coriandrum sativum L.* and *Origanum*. The third group was used as an infected group. The forth one was maintained as a control, groups 1, 2 and 3 were exposed to 50 *Schistosoma mansoni* Egyptian strain cercariae/mouse. Six weeks post challenge, TBARS measurement, reduced glutathione, IgM levels and a histopathological examination of liver sections were investigated. The results showed an increased level of TBARS (61±8.21 ng/μL) and a decrease in reduced glutathione (1.98±0.8 ng/μL) in infected mice comapred to the control ones (3.13±0.34 ng/μL). In the groups pretreated with *Nigella sativa L.* and a honey mixture, the level of reduced glutathione was observed to increase (5.96±4.20 and 12.28±2.78 ng/μL, respectively) in comparison with the untreated infected ones. TBARS measurement showed an improvement in the pretreated mice with honey of *Nigella sativa L.* (58.25±20.34 ng/μL) while a marked decrease (41.73 ng/μL) was observed upon the treatment with a honey mixture in comparison to the infected mice. Honey positively affected humoral immune response by significantly increasing IgM ($p<0.05$) upon the treatment with the two kinds of honey. Histopathological analyses revealed an improvement in liver sections in two variants of honey treatment as compared with the untreated infected livers. Our results may reflect potent antioxidant properties of honey against oxidative stress induced in mice after *S. mansoni* infection.

## INTRODUCTION

Mckibben and Engeseth [2002] and Antony *et al.* [2000] have reported that honey can prevent deteriorative oxidation reactions in foods, such as lipid oxidation in meat and enzymatic browning of fruits and vegetables [Oszmiański & Lee, 1990; Chen *et al.*, 2000]. The antioxidant activity of honey varies greatly depending on the honey floral. Due to its nutritional and biological value, honey is a healthy food for both infants (*Apis mellifera* and *Apis dorsate*) and adults; honey may be compared only with human milk. The biological value of honey is the result of high energetic value, 1339kJ (320 kcal)/100 g. Honey is remarkably complex natural liquid that is reported to contain at least 181 substances [Nele *et al.*, 2002]. Apart from carbohydrates, honey contains a small amount of proteins, free amino acids, vitamins, organic acids, salicylic acid [Venema *et al.*, 1996] and mineral substances [Scheier, 2001]. It also contains biologically--active substances such as anthocyanins, leucoailocyanins and catechols [Nele *et al.*, 2002]. Honey has been used as a medicine through the ages and in more recent times it has been rediscovered by the medical profession as a natural antibiotic agent, since five antibiotic compounds have been separated and determined in its composition (tetracycline, oxytetragcline, doxycycline, chlootetracycline and chloranphenical). In addition, honey shows antifungal, antiprotozoal as well as preservative effects [Jovan *et al.*, 1992]. It has been proved to demonstrate an antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas* [Al Jabri *et al.*, 2003]. Moreover, due to its microbial activity, honey actively accelerates wound healing by improving the tissue and prevents infection; it may also possess an anti-inflammatory activity and stimulate the immune responses within a wound [Lusby *et al.*, 2003]. In addition, an experimental evaluation of anti-tumor properties of honey revealed its moderate and pronounced anti-metastatic effects [Klein *et al.*, 2000; Mabrouk *et al.*, 2002]. Humans can protect themselves from free radicals and reactive oxygen species (ROS) damaging compounds which have been implicated in contributing the process of aging and diseases through absorbing antioxidants from high-antioxidant foods such as honey [Schramm *et al.*, 2003; Mabrouk *et al.*, 2002].

Therefore the objective of the present work was to study the effect of antioxidative properties of honey on *Schistosoma mansoni*-infected mice.

## MATERIALS AND METHODS

**Honey.** The tested honey bee was of species *Apis mellifera* (from hamza farm – Cairo, Egypt), two types *Nigella sativa L.* and a mixture of *Origanum* and *Coriandrum sativum L.* The honey was diluted by distilled $H_2O$ (1000 mg/mL) and

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administrated intraperotenally (ip) at a daily dose of 0.5 mL for 21 days.

**Animals.** Twenty female Swiss albino mice (weighing 15–17 g) were obtained from an animal house of the National Research Center, Cairo, Egypt. They were kept in well ventilated cages and were supplied with food and water.

**Parasites.** The parasites used in this study were cercariae in the infective stage of an Egyptian strain of *Schistosoma mansoni*. They were obtained from the Theodore Bilharz Institute, Cairo, Egypt.

**Soluble worm antigen preparation (SWAP).** *Schistosoma mansoi* adult worms originated from the Theodore Bilharz Institute, Cairo, Egypt. They were obtained from experimentally-infected hamsters or mice, washed well and frozen to -70°C in a small volume of saline. The worms were frozen (-20°C) and thawed (4°C) five times, the disrupted worms were centrifuged and the supernatant was recovered, kept at 4°C overnight and the formed precipitate was removed by centrifugation at 15,000 rpm for 30 min at 4°C. The final supernatant was sterilized by filtration through a 0.45 $\mu$m Millipore membrane (Millipore, Beford, MA). Protein was determined in the sterile preparation by the method of Lowry *et al.* [1951].

**Design of the experiment.** Mice were divided into four groups (5 mice/group). The first group was treated with *Nigella sativa L.* The second group was treated with a mixture of *Cariandrum sativum L.* and *Origanum*. The third one was maintained as untreated control group. After 21 days post treatment, groups (1, 2 and 3) were subcutaneously infected with 80 cercariae per mouse [Smither & Terry, 1965] of the Egyptian strain *Schistosoma mansoni*, while the fourth group was used as a healthy control.

**Biochemical analysis.** Biochemicals used in the present study were products of high analytical grade purchased at the Sigma Chemical Company (USA).

**Measurement of reduced glutathione (ng/$\mu$L).** Reduced glutathione was measured according to Moron *et al.* [1979]. The analysis involved the extraction of GSH with metaphosphoric acid and then its reaction with dithiobisnitrobenzoic acid (DTNB) to form a yellow colour which was measured at 412 nm.

**Measurement of TBARS (ng/$\mu$L).** The evaluation of lipid peroxidation using thiobarbituric acid (TBA) was employed as one of the most frequently used and easiest methods for measurement of TBARS induced by excessive oxygen radicals. This simple method depends on measuring the small amounts of free serum malondialdehyde (MDA) in nmol/mL, formed during the process of lipid peroxidation, since it is considered as a relatively stable end product of lipid peroxidation [Esterbauer & Cheesman, 1991; Draper & Hadley, 1991].

The estimation of TBARS depends upon determination of an unsaturated solution of thiobarbituric acid reactive substance and the developed color which is measured at 532 nm of malondialdehyde according to Begona *et al.* [1994]. TBARS measurements were expressed as absorbance unit concentration (ng/$\mu$L).

**Detection of IgM level against soluble worm antigen preparation (SWAP) using enzyme linked immunosorbent assay (ELISA).** The assay was performed according to Hillyer *et al.* [1979]. The assay was used to determine an IgM level in different experimental groups. Blood was collected and allowed to clot. Sera were obtained by centrifugation and stored at -20°C until use. Wells of microtiter plate (Immunol -4-dyndatch, USA) were coated with 50 $\mu$g/mL of SWAP in carbonate buffer (pH 9.6) and incubated overnight at 4°C. The plates were washed with phosphate buffer saline containing 0.05%Tween-20 (PBS-T-20, pH 7.2) and blocked for sites free of serum albumin in PBS--T20. The sera (100 $\mu$L/well) were diluted (1:100) in PBS--T.20, then added and incubated at 37°C for 2 h. After washing, antimouse IgM peroxidase conjugate (Sigma, Co.) was added 100 $\mu$L/well at a dilution of 1:10000 in PBS-0.05% T-1% B.S.A and incubated for 1 h at 37°C. Orthophenylene diamine dihydrochloride (OPD) was used as a substrate. The plate was read at $\lambda$max 492 nm with a micro-well plate reader (TECAN-SUNRISE, Austria).

**Histological analysis.** Based on the method used by Afifi [1986], use was made of the histological technique. Livers were removed from mice of the experimental groups. Pieces displaying macroscopic abnormalities were fixed in 10% formaldehyde. Sections were stained with haematoxylin-Eosin. The stained sections were observed under a light microscope at different magnifications.

## RESULTS AND DISCUSSION

It was noted that the mean level of reduced glutathione was significantly increased in rats pretreated with honey *Nigella sativa L.* and with a honey mixture of *Coriandrum sativum L.* and *Origanum* and infected with *Schistosoma mansoni* (5.96±4.2, 12.28±2.78 ng/$\mu$L, respectively) in comparison with the control infected group (1.98±0.8 ng/$\mu$L), (Figure 1). These results were consistent with the findings of Tonks *et al.* [2001], who reported that reactive oxygen intermediate (ROI) production was significantly decreased by pasture honey and manuka honey ($p<0.001$), and with those of Rizk [1998], who reported that the reduction in the level of reduced glutathione during the period of oxidative stress infection could be improved by an antioxidant. They also showed great compatibility with the data of Hoener *et al.* [1989], Videla *et al.* [1990], and Amaliz *et al.* [1995]. The highest level of TBARS was observed in the infected group (61 ng/$\mu$L) which showed a slight improvement in the group receiving *Nigilla sativa L*. (58.25 ng/$\mu$L) and a marked decrease in the group receiving a honey mixture (41.73 ng/$\mu$L), (Figure 1). These results are in agreement with those of Rizk [1998], who reported that high oxidative stress effects induced by bilharzial infestation led to free radical generation. The present study showed that the two pretreated groups markedly recorded an obvious decrease in the mal-

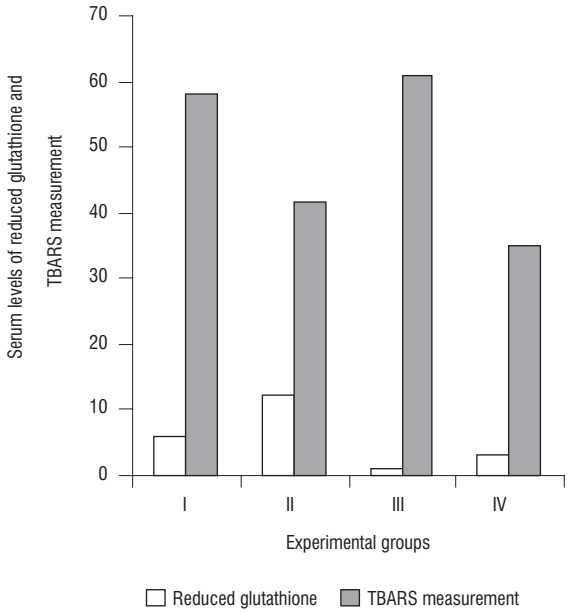


FIGURE 1. Detection of reduced glutathione and TBARS measurement (ng/$\mu$L) in sera from mice infected with *Schistosoma mansoni* and pre-treated with *Nigella sativa L.* honey (group I), pre-treated with a mixture of *Origanum* and *Coriandrum sativa L*. honey (group II), non-treated infected positive control group (III), and non-treated non-infected normal control (group IV).

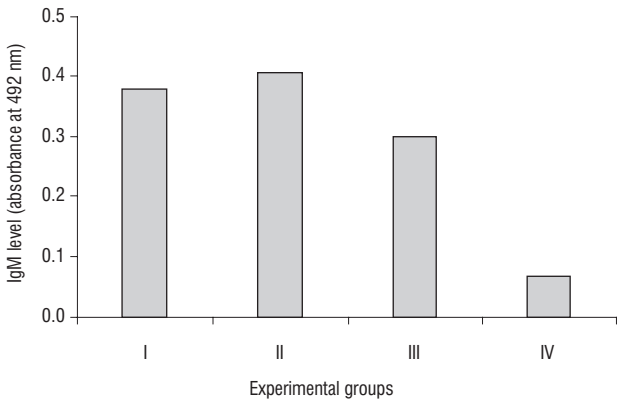


FIGURE 2. Detection of IgM response against SWAP in sera from mice treated with *Nigella sativa L.* honey and infected with *Schistosoma mansoni* (group I), mice treated with a mixture of *Origanum* and *Coriandrum sativa* L. honey and infected with *Schistosoma mansoni* (group II), non-treated and infected with *Schistosoma mansoni* positive control (group III), and non-treated and non-infected normal control (group IV).

ondialdehyde level within or near the normal level, especially in the group receiving a mixture of two kinds of honey, which strongly indicates the collective antioxidant potency of honey from different sources.

Moreover, Vonderplanitz [1997] and Mabrouk [1998] have previously reported that enzymes contained in honey and their precursors may empower all immune system functions. It was also recorded that IgA, IgE and IgM antibodies have all been shown to be stimulated as a result of schistosome infection [Dessaint *et al.*, 1975; Hagan *et al.*, 1998].

This was proved by the current study, since sera from mice that received *Nigella sativa* and a mixture of *Origanum* and *Coriandrum sativum L.* honey followed by *S. mansoni* infection showed a significant elevation ($p<0.05$) in the level of IgM ($0.37\pm0.01$, $0.41\pm0.01$, respectively) against SWAP when compared to the infected untreated mice ($0.27\pm0.005$) or uninfected untreated mice ($0.05\pm0.001$) (Figure 2).

The results represent the positive immunomodulatory effects of *Nigella sativa* and a mixture of *Origanum* and *Coriandrum sativum L.* honey in comparison with the infected and healthy control group.

In concerning to histopathological studies, it was noted that in the 6$^{th}$ week post infection, the granuloma formation in the infected control group was obviously developed. In addition, lymphocyte infilitrations around blood vessels and aggregation in hepatic parenchyma were noted. Worms of differentiated sex were often observed in dilated blood vessels of the portal tract. On the other hand, in the 6$^{th}$ week the granuloma of the liver of single or mixed honey groups were decreased relatively to the infected non-treated group as well as lymphocyte infiltrations and lymphatic aggregations were reduced as compared to the infected control group (Figure 3), which indicates that honey can be used as an antioxidative natural product against schistosoma infection.

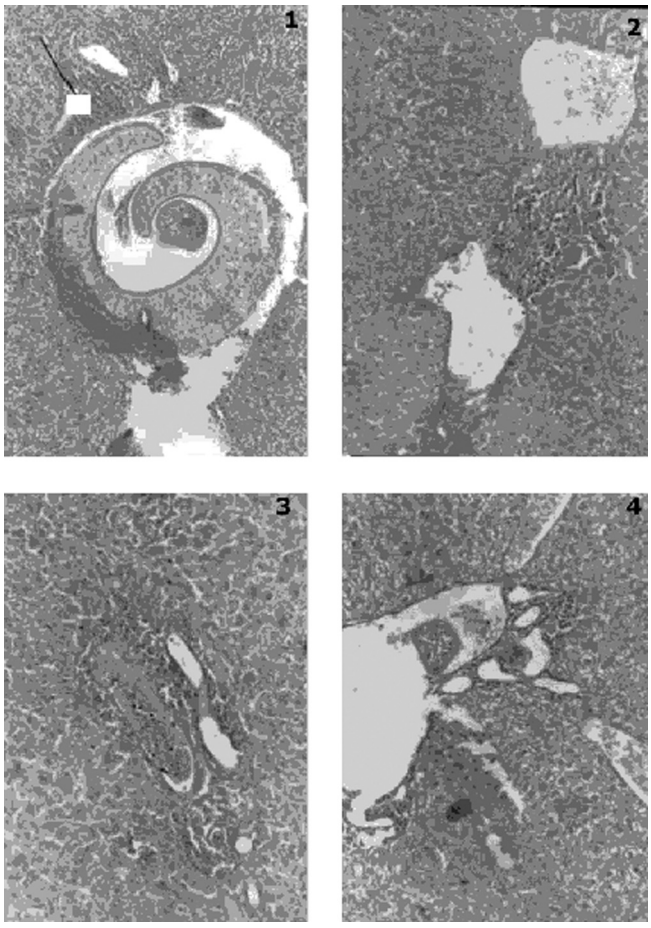


FIGURE 3. Histopathological pictures of (1) *Schistosma mansoni*-infected liver; (2) *Schistosma mansoni* pretreated with *Nigella sativa L.* where lymphocyte aggregation was observed in hepatocytes; (3) *Schistosma mansoni* pretreated with *Nigella sativa L.* where lymphocyte infiltration was observed around blood vessels; and (4) *Schistosma mansoni* pretreated with *Coriandrum sativum* L. and *Origanum* showed a small worm in blood vessels and lymphocyte infiltrations around blood vessels.

## CONCLUSIONS

The current data indicate that a mixture of *Nigella sativa L.* and *Coriandrum sativum* L and *Origanum* may demonstrate a potent antioxidant and immunoprophylactic properties, hence it is likely to increase the efficiency of the humoral immune response. It was also proved that the two types of honey can be used as antioxidants.

## REFERENCES


1. Afifi A.A., Contribution to the chemical carcinogensis in Xenopus, 1986, Ph. Thesis, UCL, Louain La Neuve, Belgium, pp. 90–91.
2. Al-Jabri A.A., Nzeako B., Al Mahrooqi Z., Al Naqdy., *In vitro* antibacterial activity of Omani and African honey. Br. J. Biomed. Sci., 2003, 60, 1–4.
3. Amaliz S.L., Llessuy S., Curtin J.C., Boveris A., Oxidative stress by acute acetaminophen administration in mouse liver. Free Radical Biol. Med., 1995, 19, 303–306.
4. Antony S., Rick J.R, Dawson P.L., Effect of dry honey on oxidation in turkey breast meat. Poult. Sci., 2000, 78, 1846–1850.
5. Begona R.L., Marina L., Merceds L., de Herbert G., Antioxidation effects of estradiol and 2-hydroxyestradiol on iron-induced lipid peroxidation of rat liver microsomes. Steroids, 1994, 59, 383–386.
6. Chen L., Mehta A., Berenbaum M., Zangerl A.R., Engeseth N.J., Honeys from different floral sources as inhibitors of enzymatic browning in fruit and vegetable homogenates. J. Agric. Food Chem., 2000, 48, 4997–5000.
7. Dessaint J.P., Capron M., Bout D., Capron A., Quantitative determination of specific IgE antibodies to schistosome antigens and serum IgE levels in patients with schistosomiasis. Clin. Exp. Immunol., 1975, 20, 427.
8. Draper H.H., Hadley M., Malondialdehyde determination as index of lipid peroxidation. 1991, *in*: Methods of Enzymology, vol. 186 (eds. L. Parcker, A. Glazer). New York, Academic Press, pp. 421–431.
9. Esterbauer H., Cheesman K.H., Determination of aldehydic lipid peroxidation products: MDA and hydroxymonenal. 1991, *in*: Methods of Enzymology, vol. 186 (eds. L. Parcker, A. Glazer). New York, Academic Press, pp. 407–421.
10. Hagan P., El-Meleigy M. Traor M., Schistosomiasis Research: The end of the beginning. Parasitol. Today, 1998, 111, 392–394.
11. Hillyer G.V., Gomez D.R.I., The enzyme linked immunosorbent assay (ELISA) for the immunodiagnosis of schistosomiasis. Am. J. Trop. Med. Hyg., 1979, 28, 237–241.
12. Hoener B.N., Andrup M., Yen T.B., Nitrofurantoin produces oxidative stress and loss of glutathione and protein thiols in the isolated perfused rat liver. Pharmacology, 1989, 38, 363.
13. Jovan V., Ivanka K. Mileta J., Chemical composition, biological value and application of honey. Hem. Pregl., 1992, 33, 3–4.
14. Klein C., Sato T., Meguid M.M., Miyata G., Honey has proven antimicrobial activity. J. Gastroenterol., 2000, 35, 1–6.
15. Lowry O.H., Rosebrough N.H., Farr A.L., Tandall R.J., Protein measurement with the Folin Phenol reagent. J. Biol. Chem., 1951, 193, 265.
16. Lusby P.E., Coombes A., Wilkinson J.M., Honey: a potent agent for wound healing. J. Wound Ostomy Continence Nurs., 2003, 29, 295–300.
17. Mabrouk G.M., Moselhy S.S., Zohny S.F., Ali E.M., Helal T.E., Amin A.A., McKenna J., Natural Alternatives to Antibiotics: Using Nature's Pharmacy to Help Fight Infections. 1998, Garden City Park, NK: Avery Publishing Group, p. 10.
18. Mabrouk G.M., Moselhy S.S., Zohmy S.F., Ali E.M., Helal T.E., Amin A.A., Khalifa A.A., Inhibition of methylnitrosourea induced oxidative stress and carcinogenesis by orally administered bee honey and *Nigella* grains in Sprague Dawely rats. J. Exp. Clin. Cancer Res., 2002, 21, 341–346.
19. Mckibben J., Engeseth N.J., Honey as a protective agent against lipid oxidation in ground turkey. J. Agric. Food Chem., 2002, 50, 592–595.
20. Moron M.S., Depierre J.W., Mannervik, B., Levels of glutathione, glutathione reductase and glutathione–s transferase activities in rat lung and liver. Biochem. Biophys. Acta, 1979, 582, 67–78.
21. Nele G., Xiao-Hong W., Nicki J.E., Identification and quantification of antioxidants of honeys from various floral sources. J. Agric. Food Chem., 2002, 50, 5870–5877.
22. Oszmianski J., Lee C.Y., Inhibition of polyphenol oxidase activity and browning by honey. J. Agric. Food Chem., 1990, 38, 1892–1895.
23. Rizk M., Protective effect of *Curcuma longa* against oxidative stress in *Schistosoma mansoni*-infected mice livers. Egypt. J. Bilharzia, 1998, 21, 1–8.
24. Scheier L, Salicylic acid, one more reason to eat your fruits and vegetables. J. Am. Diet. Assoc., 2001, 101, 1406–1408 .
25. Schramm D.D., Karim M., Schrader H.R., Holt R.R., Cardetti M., Keen C.L., Honey with high levels of antioxidants can provide protection to healthy human subjects. J. Agric. Food Chem., 2003, 1251, 1732–1735.
26. Smithers S.R., Terry R.J., The infection of laboratory hosts with cercariae of *Schistosoma mansoni* and the recovery of adult worms. J. Parasitology, 1965, 55, 695–700.
27. Tonks A., Cooper R.A., Price A.J., Modan P.C., Jones K.P., Stimulation of TNF. Alpha released in monocytes by honey. Cytokine, 2001, 14, 240–242.
28. Venema D.P., Hollman P.C.H., Janssen K.P.L.T.M., Katan M.B., Determination of acetylsalicylic acid and salicylic acid in foods, using HPLC with fluorescence detection. J. Agric. Food Chem., 1996, 44, 1762–1767.
29. Videla L.A., Barros S.B.M., Jnqueira V.B.C., Lindana-induced liver oxidative stress: free radical. Biol. Med., 1990, 9, 169.
30. Vonderplanitz A., We want to live. 1997, *in*: Out of the Grips of Disease and Death. Santa Monica, Carnelian Bay Castle Press, p. 149.


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