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EFFECT OF BLACK SEED OIL ON RABBITS INFECTED WITH SOME INTESTINAL EIMERIA SPECIES

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SUMMARY

Black seed (*Nigella sativa*) is a herb used on large scale in veterinary practice for many purposes. The protective effect of black seed oil was studied on rabbits experimentally infected with some field strains of intestinal *Eimeria* species. For this purpose, 30 New-Zealand rabbits, 28 days old were divided into 6 groups. Group (1) was used as prophylactic, given *N. sativa* oil at a dose of 2.5 ml/ kg body weight orally for 3 weeks daily, then challenged with 6 x 10^4 Eimeria oocysts. Group (2) was treated with sulphadimidine $33^1/_3$% after being infected. Group (3) was treated as group (2), in addition to *N.sativa* oil for 2 weeks. Group (4) was as infected control one, while group (5)) was given *N. sativa* oil only. Group (6) was non infected and non treated. The results revealed a significant decrease of Eimeria oocysts in faeces of both prophylactic and treated groups. However, there were increase in phagocytic activities of neutrophils expressed in phagocytic percent and phagocytic index. Furthermore, *N. sativa* oil improved body weight gain and increased serum total protein, albumin and Albumin /Globulin ratio in treated rabbits.

So, it was concluded that, *N. sativa* seeds which is cheap local plant, can be used as immune enhancing factor and growth stimulant in ration of animal.

INTRODUCTION

Rabbits have an economic importance either as a source of high quality protein of meat, fur production and used as laboratory animals. Many factors affect rabbit industry such as morbidity

nd mortality due to viral, bacterial, and parasitic nfections. Coccidiosis is an important protozoal nfection resulting in great losses due to death, oss of weight and expense of treatment (Haiba et l. 1965). More than eleven Eimeria species were escribed from domestic rabbits, six of which are nore pathogenic and cause severe coccidiosis nd death (Pellerdy, 1974).

The use of anti coccidial medication on regular ases leads to development of drug resistance McDougald, 1990) and damage of some organs s kidney and liver, as well as residues of anti-occidial drugs that could present in rabbit meat vith its undesirable effect on human health (Atta nd EL- Zeni, 1999).

o, the need of alternative medicine is required uch as herbal therapy which may help either by reatment or by increasing the immune response o the disease.

Many reports have been focused on the biological ctivities of black seeds (Nigella sativa) including mmunopotentiation, antioxidant, hypoglycaemia, nd antibacterial effects (Singab and Okuyama, 001) Nigella sative oil has been used in veteri-ary practice for control of tape worms in sheep Agarwal et al. 1979), against cryptosporidium in nice (El-Refaii, 2003), and as anticoccidial in chicken (El- sayed and El- Hashem, 2000).

The distinct success of N. sativa stimulate us to evaluate it as immune stimulant and antiparasitic agent on rabbits experimentally infected with some intestinal Eimeria.

MATERIALS AND METHODS

I- Experimental animals:

A total number of thirty healthy, parasite- free New Zealand rabbits, 28 day old were used. Rabbits were divided into six groups, each of five animals, kept individually in cages under coccidia free housing condition.

A commercial pelleted feed and water were added ad libitum. Animal body weights were recorded before and after the experiment.

II- Parasites:

Eimeria oocysts used for infection were recently sporulated and kept at 4°C in 2.5% potassium dichromate water solution. The species of parasites were obtained from 38 naturally infected rabbits and identified according to Soulsby (1982). Sporulated Eimeria oocysts were counted using modified McMaster technique.

III- Black seed oil:

It was obtained from private seller and administrated orally at a dose of 2.5 ml/ kg body weight of rabbit daily, according to Mahmoud et al (2002).

IV- Experimental design:

Rabbits of group (1) were used for prophylactic purpose, they were given Black seed oil for 3 weeks daily, then inoculated per os with 6 x 10^4 sporulated oocysts of the prepared mixed Eimeria species. The method used for counting was that of Coudert et al (1995).

Rabbits of groups (2) and (3) were used for treatment purpose; they were infected with Eimeria oocyst at the same time of group (1). Then treated after presence of the symptoms of infection (2 weeks). Group (2) was treated with 0.5 ml subcutaneous injection of 33 1/3 % sulphadimidine sodium (Vetwic, El- Nasr Pharmaceutical Chemical Co. Egypt.) for 5 days.

Group (3) was treated as group (2), in addition to oral administration of Black seed oil for 2 weeks.

Rabbits of group (4) were used as control infected animal, given sporulated Eimeria oocysts only.

Rabbits of group (5) was administrated B.S oil for 3 weeks.

Rabbits of group (6) was used as control non infected, non treated animals.

Faecal samples from each rabbit were examined daily using concentration floatation technique every week, and total oocyst count was done using modified Mc Master technique. Two blood samples from each animal were obtained 28 days after infection, the first sample was taken in dry tubes for serum separation to estimate serum total protein and albumin according to Henry (1968) and Dumas et al. (1971) respectively. Globulin contents was calculated by subtraction of albumin from total protein. The second blood sample was taken on heparin (20 i.u./ml blood) for detection of phagocytic activities of neutrophils using Candida albicans according to method described by. Wilkinson (1981).

Statistical analysis:

Data obtained were statistically analysed using analysis of variance and comparing between groups were performed using least significant difference (LSD) at $P < 0.05$ according to Petrie and Watson (1999) and computerized using SPSS (2002).

RESULTS

I- Parasitological findings:

Four Eimeria species were isolated from 38 rabbits, *E. magna, E. intestinalis, E. coecicola,* and *E. perforans* (Fig., 2 and table, 1). Faecal examination indicated a significant decrease in Eimeria oocysts count in groups (1, 2, 3) as compared with control group (4), 28 days post infection as shown in table (2).

II- Body weight gain:

Table (3) showed an improved body weight gain in group (5) as compared with group (6). Also, body weight gain was improved in groups (1, 2, 3) as compared with group (4).

III- Biochemical results:

The results in table (4) showed that serum total protein, albumin and A/G ratio were significantly increased in rabbits given Black seed oil (group 5) as compared with group (6).

There is a significant decrease in these parameters in the control infected group (4), but groups (1, 2, 3) were less affected by coccidial infection.

Serum globulin contents were increased in gro (4).

IV- Immunological results:

Phagocytic activity of neutrophils as shown in ble (5) and Fig. (1) revealed highly significant crease in phagocytic percentage and phagocy index in group (5), and a significant decrease group (4) as compared with groups (1, 2 & 3).

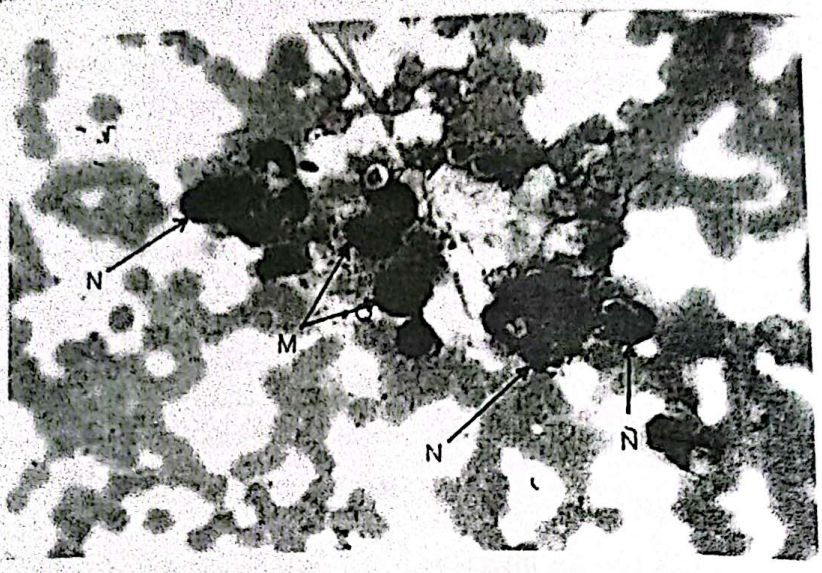

Fig. (1): Peripheral blood neutrophils engulfing Candida albicans (Giemsa stain x 1000).
M= Monocytes. N= Neutrophils

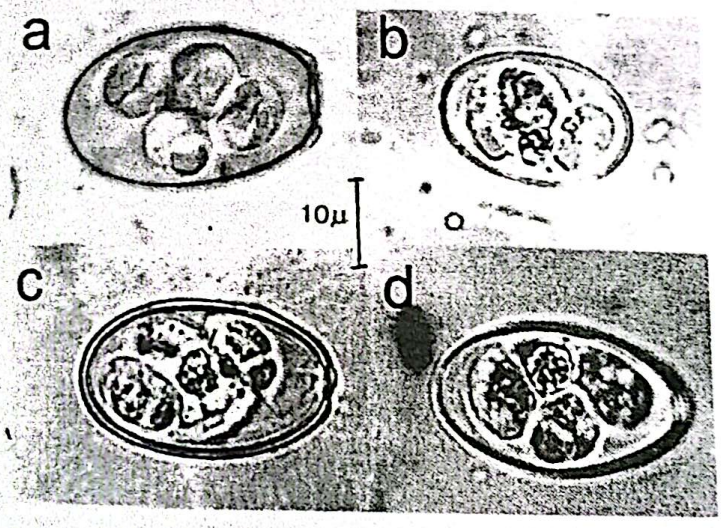

Fig. (2): Sporulated oocysts of *E. magna* (a), *E. perforans* (b), *E. coecicola* (c), and *E. intestinalis* (d) (x 1000).

Table (1): Morphological features of isolated Eimeria species oocysts.

	E. magna	*E. perforans*	*E. coecicola*	*E. intestinalis*
Sporulation Time	2-3 days	1-2 days	3 days	1-2 days
Shape	Ovoid	Ovoid	Cylindrical	Pyriform
Colour	Yellowish brown	Light pink	Bright yellow	Yellowish
Length	28-40 (35 µ)	15-29 (22.7 µ)	25-39 (34 µ)	22-33 (27 µ)
Breadth	22-26 (24 µ)	11-17 (14.2 µ)	16-22 (19 µ)	16-21 (18 µ)
Micropyle	5-7 (6.2 µ)	--	4-6 (5.5 µ)	5-6 (5.4 µ)
Residual body	8-14 (10.3 µ)	6-9 (7.2 µ)	6-8 (6.4 µ)	6-9 (8.1 µ)

Table (2): Eimeria oocysts output / gm faeces (10^3) of different experimental groups, at different periods.

Days post-infection	GP. 1 Mean ± S. E	GP. 2 Mean ± S. E	GP. 3 Mean ± S. E	GP. 4 Mean ± S. E	GP. 5Mean ± S. E	GP. 6Mean ± S. E	F. calculated
14	0.47 ± 0.021 ^{A}	0.68 ± 0.031^{a}	0.69 ± 0.023^{a}	0.67± 0.031^{a}	-	-	15.666#
21	2.52 ± 0.031 ^{A}	5.76 ± 0.026 ^{a}	5.70 ± 0.027 ^{aB}	5.80 ± 0.037^{ab}	-	-	2782.243#
28	3.008 ± 0.1618^{A}	0.500 ± 0.0141^{aB}	0.100 ± 0.0140 ^{abC}	12.300± 0.0192 ^{abc}	-	-	4839.393 #

Significant at P > 0.05 using ANOVA Aa, Bb, Cc significantly different between two comparison groups against capital litters using LSD at P > 0.05.

Table (3): Average body weight (gm), and mortality rate percentage in different experimental groups.

Body wt and mortality rate	GP. 1	GP. 2	GP. 3	GP. 4	GP. 5	GP. 6	F. calculated
28 days old	541 ± 4.96	549 ± 40.34	544 ± 6.97	547 ± 2.49	537 ± 12.30	540 ± 4.45	0.479
56 days old	1512 ± 4.66 ^{A}	1265 ± 15.84^{aB}	493± 4.78 ^{bC}	1127± 5.60^{acD}	1564 ± 9.15^{bcdE}	1500± 39.04^{bdc}	93.137 #
Mortality rate	0%	0%	0%	40%	0%	0%	-

Significant at P > 0.05 using ANOVA Aa, Bb, Cc significantly different between two comparison groups against capital litters using LSD at P > 0.05.

Table (4): Total serum protein, albumin, globulin (gm/dl), and A/G ratio of different groups of rabbits.

Group / Parameters	GP. 1	GP. 2	GP. 3	GP. 4	GP. 5	GP. 6	F. calculated
Total Protein	5.67 ± 0.11^{A}	5.70 ± 0.07^{B}	5.70 ± 0.05^{C}	5.20 ± 0.11^{abcD}	6.20± 0.05^{abcdE}	5.70 ± 0.22^{dc}	7.31#
Albumin	2.9 ± 0.04^{A}	3.0 ± 0.02^{ab}	3.2 ± 0.03^{abC}	2.3 ± 0.04^{abcD}	3.7 ±0.07^{abcdE}	3.4 ± 0.03^{abcdE}	167.24#
Globulin	2.6 ± 0.11	2.6 ± 0.06	2.5 ± 0.07^{A}	2.9 ± 0.11^{ab}	2.5 ± 0.08^{b}	2.4 ± 0.2^{b}	1.50#
Albumin/ globulin ratio (A/G	1.1 ± 0.05	1.2 ± 0.02	1.3 ± 0.04	0.8 ± 0.03	1.4 ± 0.05	1.4 ± 0.1	11.37#

Significant at p> 0.05 using ANOVA test.

Aa, Bb, Cc, Dd, Ec significantly different between two comparison groups against capital litters using LSD at P > 0.05.

Table (5): Phagocytic percentage and phagocytic index of rabbit neutrophils of different experimental groups.

Parameters \ Group	GP. 1	GP. 2	GP. 3	GP. 4	GP. 5	GP. 6	F. calculated
Phagocytic %	72 ± 1.0 ^{A}	55 ± 2.5^{aB}	65 ± 1.0 ^{abC}	53 ± 1.2^{acD}	77 ± 1.7^{abcdE}	63 ± 2.0^{abde}	54.24# 11.08#
Phagocytic Index	1.3 ± 0.06	1.0 ± 0.04	1.2 ± 0.04	1.0 ± 0.02	1.4 ± 0.04	1.2 ± 0.02	

Significant at P > 0.05 using ANOVA test.

Aa, Bb, Cc, Dd, Ee significantly different between two comparison groups against capital litters using LSD at P > 0.05.

$$\text{Phagocytic \%} = \frac{\text{number of neutrophils ingesting Candida albican X100}}{\text{Total number of neutrophils}}$$

$$\text{Phagocytic Index} = \frac{\text{Total number of Candida albican ingested by neutrophils}}{\text{Total number of neutrophils ingesting Candida albican}}$$

DISCUSSION

In this work, *N. sativa* oil was used to study its immune- enhancing and therapeutic effect on rabbits infected with some Eimeria species.

In case of prophylactic group (1), it was evident that *N. sative* gave a great support for infected rabbits to tolerate the infection with coccidia, and this was reflected in a significant decrease in faecal oocyst output, significant increase in phagocytic percentage and phagocytic index, in addition to absence of mortalities.

These results might be attributed to the effect of *N. sativa* on the immune system of rabbits, and its mechanism of action seemed to be complex. Khaled et al. (1988), mentioned that the protective effect of N. sativa by increasing both humoral and cell- mediated immune response.

The immune cells of the intestinal mucosa includes thymus derived lymphocytes or T- cells (CD4+ and CD8+), in addition to B- cells, natural killer cells and phagocytic cells (McDonald, 1999). Both CD4+ and CD8+ T- cells appear to be important in the immune response against in-

tra- cellular protozoa including Eimeria (Lillehoj and Trout, 1996). Neutrophils and natural killer cells are two cell- types responsible for the protective immune response during primary infections (Schito and Barta, 1997). *N. sativa* enhances the cellular immune- response of B and T-cells, activates macrophages and natural killer cells (Basil and Hashim, 1993).

The immune potentiating effect *N. sativa* was mediated through stimulation of macrophage phagocytic activity either directly or via activation of lymphocytes (Fararh et al., 2004).

Furthermore, Black seed oil, increased macrophage number and function and gamma- interferon production. The activation of neutrophils can be enhanced by gamma- interferon (Tizard, 1996 and Salem and Hossain, 2000).

Group (3), was treated with both sulphadimidine and N. sativa oil and gave better results more than group (2) which was treated with sulphadimidine only. This indicates that the oil potentiate the effect of the drug and contain antiprotozoal effect. Similar results were obtained by Wahba (2002) and El- Refaii (2003).

The infected control group (4) showed a significant increase in faecal oocyst count, 40% mortality, very poor body weight and severe pathological lesions in the intestinal tract due to severe coccidiosis. These results agree with those obtained by Ahmed (2005). Serum total protein, albumin and A/G ratio were significantly reduced, while serum globulin was increased. The hypo proteinaemia was also recoded by EL-Masry (2003), the significant increase in serum globulin may be due to increase of gamma- globulin as a result of greater response of host to a stronger development of the parasites (GomezñBautista et al., 1986), in addition, the increase production of lymphocytes will in turn increase the immune-globulin (Abd El-Aal and Attia, 1993).

In case of treated group (5) which was given N. sativa oil only showed good health condition with increased growth rate, serum total protein, albumin and A/G ratio. The high body gain may be due to that *N. sativa* oil increased food consumption and feed conversion rate (Brander 1982), and that extract contain more than 100 components which worked synergistically, and rich in poly unsaturated fatty acids which are the building blocks of cells, protein which sustained body health, as well as thymohydroquinone which was proved to have antimicrobial effect (El- Fatatry, 1975).

The anti microbial properties of N. sativa components, treated the subclinical cases and improving the general health of the animal (Rathee et al., 1982).

The increase of serum total protein and albumin might be attributed to the high percentage of

ude protein contents (20.5%) of N. sativa (Abd l- Aal and Attia, 1993), and due to the direct effect of N. sativa on haemopioetic tissues (Khary et al., 1996). So, it was concluded that N. sativa which is cheap local plant, can be used successfully as immune enhancing factor and growth stimulant especially in animals of lowered immunity against certain diseases.

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تأثير زيت الحبة السوداء على الأرانب المصابة ببعض أنواع الكوكسيديا المعوية

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نبات الحبة السوداء أو حبة البركة ، من الأعشاب الطبية واسعة الإنتشار والمستخدمة فى أغراض متعددة فى الم البيطرى.

أجريت هذه الدراسة لمعرفة التأثير الوقائى لزيت الحبة السوداء على مجموعة الأرانب المصابة معملياً ببعض أنو الكوكسيديا المعوية. لهذا الغرض تم إستخادم عدد ٣٠ أرنب من نوع البوسكات الأبيض عمر ٢٨ يوماً ، وقد تم تقسيم الأرا إلى ٦ مجموعات. المجموعة الأولى أعطيت ٢.٥ ملى زيت بالفم لكل كجم من وزن الأرنب يومياً لمدة ثلاثة أسابيع، ثم أعط (6×10^4) حويصلة كوكسيديا.

المجموعة الثانية أعطيت نفس العدد من الحويصلات ثم تم علاجها بعقار السلفا ديميدين $3/1$٣٣٪ ، والمجموعة الثالثة علاجها مثل المجموعة الثانية بالإضافة إلى زيت الحبة السوداء.

المجموعة الرابعة هى الضابطة المصابة، والمجموعة الخامسة أعطيت زيت الحبة السوداء فقط ثم المجموعة السادسة هى الضابطة الغير المصابة.

إجريت الإختبارات الطفيلية والبيوكيماوية والباثولوجية والمناعية على المجموعات الستة وتم تحليل النتائج إحصائياً.

وقد تبين من نتائج الفحوص المختلفة أن إستعمال زيت الحبة السوداء يقلل من شدة الإصابة وعدد حويصلات الكوكسيديا الخارجة مع البراز، كما أنه يزيد من نشاط وعدد الخلايا المناعية.

وأظهرت الدراسة أيضاً زيادة معدل النمو وكمية البرتين الكلى وسابق الزلال فى دم الأرانب المعالجة بزيت الحبة السوداء.

ويتضح من هذه الدراسة أن لنبات الحبة البركة تأثيره الواضح فى الوقاية والعلاج ويمكن إستخدامه كإضافة غذائية فى علائق الحيوان.