ELECTRON MICROSCOPICAL STUDIES ON THE EFFECT OF NIGELLA SATIVA SUPPLEMENTATION ON SWISS MICE EXPERIMENTALLY INFECTION WITH *SALMONELLA TYPHIMURIUM*

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Abstract

This work has been designed to investigate possible effects of Nigella sativa supplementation on the mice experimentally infected with *Salmonella Typhimurium* using the electron microscope. The mice that took diet with Nigella sativa and experimentally infected with *Salmonella Typhimurium* showed survival rate of 96% compared with the group that had diet without Nigella sativa. Also, electron microscopical observation revealed that mice treated group with Nigella sativa showed few changes in the liver as slight mitochondria swelling and less cytoplasmic and nuclear alteration, while, those of non-treated group showed changes included swelling of mitochondria, proliferation of rough endoplamic reticulum, cytoplasmic condensation and nuclear degeneration, dilatation of sinusoid and intracellular space.

INTRODUCTION

Mice are susceptible to salmonellosis specially *Salmonella Typhimurium,* and significant economic losses occurred due to high mortality rates, in which the immune deficiency status plays an important role in the progress of the bacteria (Pietro and Duncan, 2006).

Furthermore, the possibility of preventing the disease using dietary supplementation and/or herbal medicine attracted considerable attension (Rofaiil, 2007).

Nutrition has a profound effect on immunity and health of animals, the nutrition deficiencies impair the immune responsiveness and thereby increase mortality and subsequently the production capacity (*James et al.,* 2002).

During the last years, great attention was directed to the use of therapeutic agents of plant origin against many infections. Nigella sativa is a herb native to the Mediterranean region that belongs to the family Ranunculeceae. The active principle of Nigella sativa was found to inhibit the growth of several species of pathogenic organisms and also has a beneficial effect on the immune system (Wahba, 2002, El-Kalash and Wahba, 2005, Rofaiil and Daoud, 2005).

Several studies have been published only at the light microscopical level on the effect of Nigella sativa on microbial agents (El-Refaii, 2003, El-Kalash and Wahba, 2005). But, little effort was undertaken to study the electron microscopical investigation of such effect.

Thus, this study was carried out to determine the histopathological changes in the liver of mice fed Nigella sativa using the electron microscope.

MATERIALS AND METHODS

Experimental mice

One hundred and fifty mice 18-20 g were examined to be serologically and bacteriologically free from *Salmonella Typhimurium.* They were kept in separated cages under strict hygienic conditions and fed on balanced ration during the experiment.

Ration

A balanced ration was examined bacteriologically and proved to be free from salmonella.

Nigella sativa seeds

They were obtained from commercial source, crushed and thoroughly mixed with 2.5 % of basal ration.

***Salmonella Typhimurium* strain**

A local isolate of *Salmonella Typhimurium* was confirmed through Gram staining, colonial morphology, biochemical and serological reaction as described by Forbes *et al.* (1998).

Experimental design

All mice were divided into 3 groups, 50 mice each, and were treated as follows:

Mice of group (1) were fed on commercial ration with Nigella sativa crushed seeds at a concentration of 2.5%.

Mice of group (2) were fed on commercial ration without Nigella sativa supplementation.

Mice of group (3) were left as control group, had a normal ration without Nigella sativa and not experimentally infected with Salmonella Typhimurium.

The mice of groups 1 and 2 were experimentally injected intraperitoneally with 0.2 ml twenty - four hours broth culture of *Salmonella Typhimurium* containing 1.5 x 10^8 cfu/ml. Such mice were observed daily after experimental infection up to 5 days to record the protection percentage.

Bacteriological examination

Smears from internal organs of freshly dead infected mice were subjected to bacteriological examination, reisolation of the organism from internal organs, and confirmatory diagnosis was done as described by Forbes *et al.* (1998).

Electron microscopy

It was performed in a guidance of Robinson *et al.* (1987). Cubes of the liver 1mm size each from all groups of mice were fixed in 5% cacodylate buffered gluteraldhyde then post fixed in 2% osmium tetraoxide, dehydrated and embedded in epon. Semi sections were obtained and stained with 0.25% Toulidine blue for selection of suitable areas by light microscopy, ultra thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate and examined by TEM (100 x IJ, Joel, Tokyo, Japan) at 80 KV.

RESULTS AND DISCUSSION

This work has been designed to determine the histopathological changes in the liver of mice fed Nigella sativa using the electron microscope.

Data recorded in Table 1 showed that mice that received Nigella sativa in their ration resisted infection with *Salmonella typhimurium* and the percentage of their survival rate reached 96% compared to 20% of the non-treated group.

From the obtained results, the anti-microbial response produced by feeding mice on ration supplemented with Nigella sativa is similar to that obtained by Wahba (2002) who found that rats treated with Nigella sativa could tolerate the infection, while, those non-treated did not survive. Also, El-Refaii (2003) reported that Nigella sativa had a therapeutic effect on *Cryptosporidium parvum* in experimentally infected mice and found that treated group with Nigella sativa showed a reduction in oocysts count, when compared to those non-treated group.

The ultra-structural changes in group 2 experimentally infected with *Salmonella Typhimurium* and not supplemented with Nigella sativa, were in the form of mitochondria swelling (Fig. 1), proliferation of the rough endoplasmic reticulum (Fig. 2), cytoplasmic condensation of hepatocytes (Fig. 3), nuclear degeneration and fragmentation of hepatocytes (Fig. 4), and intracellular space dilated sinusoid (Fig. 5). In group (2) which took Nigella sativa supplementation with ration and experimentally infected with *Salmonella typhimurium*, the electron microscopical examination showed few changes compared with those infected with *Salmonella typhimurium* only. These changes were slight mitochondria swelling (Fig. 6), less cytoplasmic and nuclear alteration (Fig. 7), and clear intact sinusoid (Fig. 8). (Fig. 9) illustrates the ultra-structure of normal liver.

The ultrastructural changes observed in liver of mice experimentally infected with *Salmonella typhimurium* as illustrated in Figs. 1-5 confirm with those recorded by Maccky (1981), Ghadially (1997) and Nasser (1999).

The administration of Nigella sativa may induce marked improvement in diminishing the severity of alteration in the affected liver. Also, several investigations which were carried out concerning the protective effect of Nigella sativa against variety of pathogens (Wahba, 2002, El-Refaii, 2003 and Rofaiil and Daoud, 2005) reported that Nigella sativa has an immunostimulatory effect, it stimulated the immune system and decreased the lethal effect of *Salmonella typhimurium* in the infected mice compared to those of control group.

Moreover, El-Ballal and Manakhly (1998) suggested that administration of Nigella sativa extracts at a therapeutic level may minimize the neurotoxic potential acrylamide in rats at ultrastructural evaluation. Such results were also obtained by Abdel Aziz *et al.* (1995), who explained that the improvement of the immune status of mice, is due to the enhancement of the natural killer cells to restore their activity.

From the pathological point of view, the group of mice supplemented with Nigella sativa in ration has tolerated the intraperitoneal inoculation with *Salmonella typhimurium*. The liver, by electron microscopical investigation appeared with less changes. This observation is in agreement with that recorded by Wisse *et al.* (1994).

The control group showed normal ultrastructural feature of liver, and *Salmonella typhimurium* could induce a characteristic ultrastructural changes on experimental infection of mice, while, Nigella sativa reduced these changes. So, it could be concluded that continuous administration of Nigella sativa at a protective level minimizes the infection of mice and could be used as feed additive.

Table 1. Efficacy of Nigella sativa supplementation in ration of mice experimentally infected with *Salmonella typhimurium*.

Groups of mice	No. of dead mice/Total No. of mice	Mortality rate	No. of survived mice/Total No. of mice	%
Group (1)	2/50	4 %	48/50	96
Group (2)	40/50	80 %	10/50	20
Group (3)	0/50	0 %	50/50	100

Group1. Supplemented with Nigella sativa in ration and infected with Salmonella *typhimurium*.

Group 2. Not supplemented with Nigella sativa in ration and infected with Salmonella Typhimurium.

Group 3. Control not supplemented with Nigella sativa in ration and not infected with *Salmonella typhimurium*.

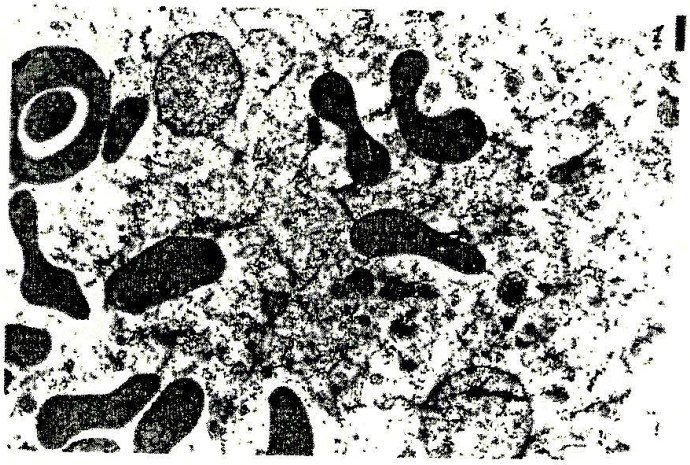

Fig. 1. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* showing severe mitochondria swelling (x8400).

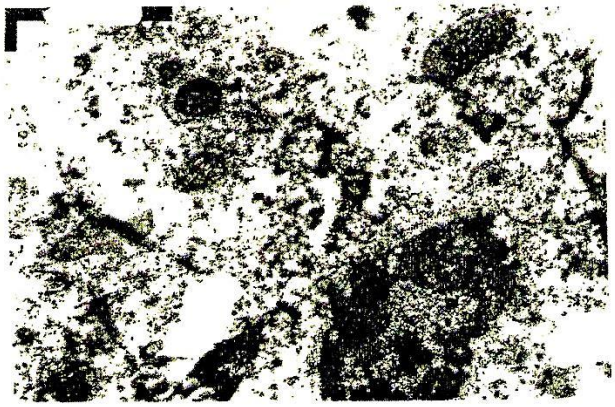

Fig. 2. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* showing proliferation of rough endoplasmic reticulum (x14000).

Fig. 3. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* showing cytoplasmic condensation of hepatocyte (x5600).

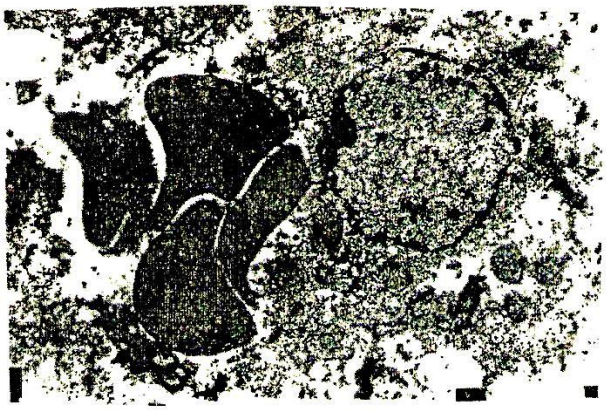

Fig. 4. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* showing nuclear degeneration and fragmentation of hepatocytes (x5600).

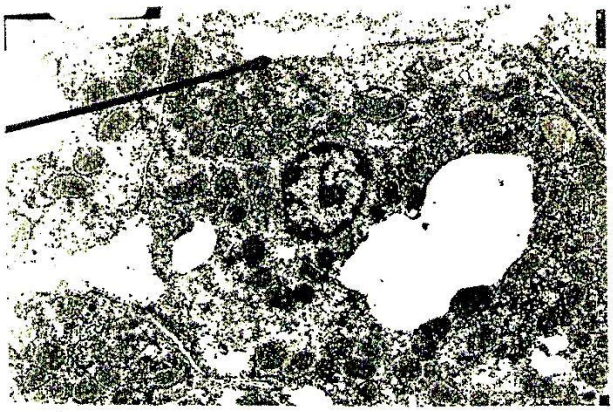

Fig. 5. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* showing intracellular space, dilated sinusoid (x5600).

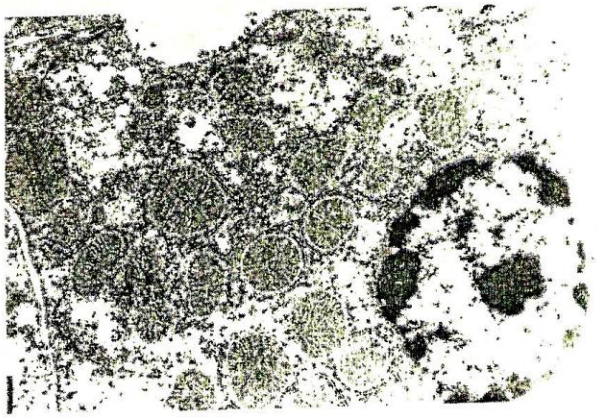

Fig. 6. Electron micrograph of the liver of mice experimentally infected with *Salmonella* typhimurium and treated with Nigella sativa showing slight degree of mitochondria swelling (x11400).

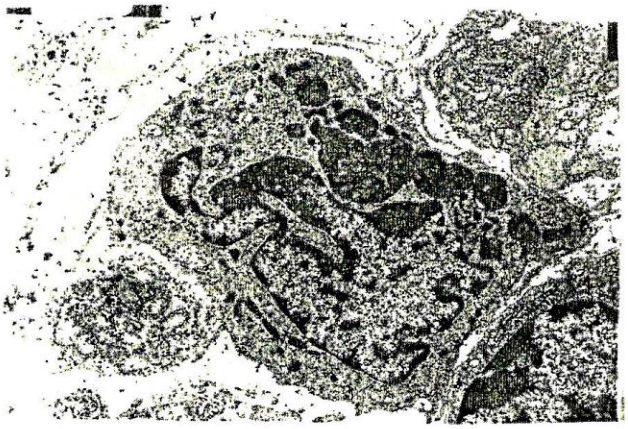

Fig. 7. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* and treated with Nigella sativa showing less cytoplasmic and nuclear alteration (x4200).

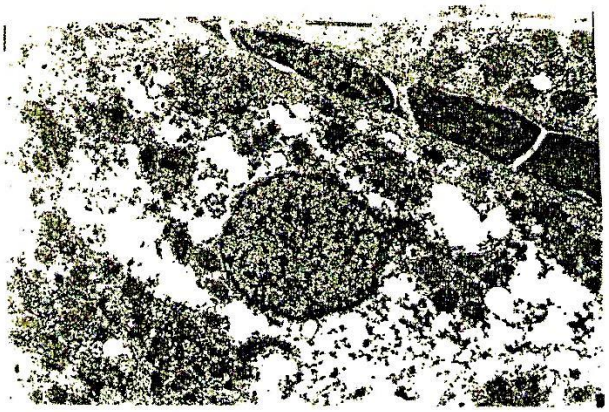

Fig. 8. Electron micrograph of the liver of mice experimentally infected with *Salmonella typhimurium* and treated with Nigella sativa showing sinusoid with intact lining endothelium (x5600).

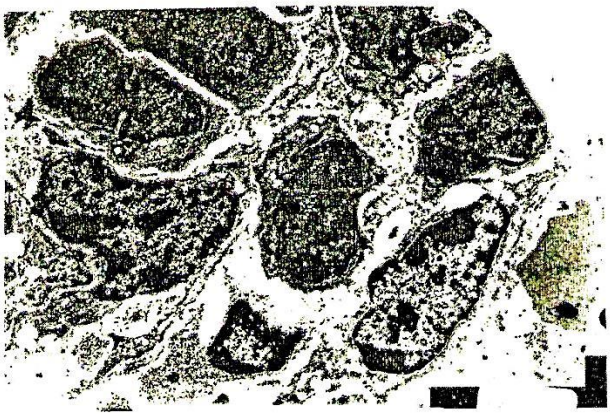

Fig. 9. Electron micrograph of the control cells showing normal ultrastructural features of hepatocytes (x5600).

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دراسات بالميكروسكوب الإلكترونى على تأثير حبة البركة على العدوى الإصطناعية بالسالمونيلا تيفيميوريوم فى الفئران السويسرية

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أجريت هذه الدراسة باستعمال الميكروسكوب الإلكترونى لمعرفة التأثير الوقائى لحبة البركة على الفئران السويسرية المعدية تجريبياً بالسالمونيلا تيفيميوريوم. وأثبتت النتائج أن هناك تأثيراً مناعياً واضحاً فى الفئران التى تم عدواها بالسالمونيلا تيفيميوريوم والمعالجة بحبة البركة حيث كانت نسبة الفئران المستمرة على قيد الحياة هى ٩٦ % بالمقارنة بالمجموعة الغير معالجة. ولم تظهر عليها تغيرات بالكبد عند فحصها بالميكروسكوب الإلكترونى سوى استطالة بسيطة فى الميتوكوندريا وتغيرات بسيطة ايضاً فى السيتوبلازم والنواة.

أما المجموعة الغير معالجة فقد ظهر بها تغيرات بالكبد عبارة عن استطالة الميتوكوندريا مع اتساع فى الشبكة الأندوبلازمية وتكثيف فى السيتوبلازم وإنحلال نووى وتوسيع المنحنى الجيبى مما يؤكد حدوث استسقاء داخل الخلايا.

ومن خلال تلك الدراسة يمكن أن يوصى بإمكانية استخدام حبة البركة فى علائق الفئران لرفع كفاءة الجهاز المناعى .