Research Article

Effect of Nigella Sativa oil on mitotic activity and liver generation in %70 partially hepatectomized rats

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ABSTRACT: Nigella sativa is a pharmacologically active quinone, which possesses several properties. This study is aimed to evalute effect of Nigella sativa on mitotic activity in partially resected liver.

Material and Methods

The study included 40 male Wistar albino rats were %70 hepatectomized and divided into four groups. On the postop 1st and 7th day tey were sacrificed. To evaluate the liver regeneration Ki-67 monoclonal antibody was used.

Results

There were statistically significant differences between Ki67 values of study and control groups. Study group have better Ki67 values.

Conclusion

We can say daily 1ml/kg nigella sativa oil increase mitotic rate and regenaration capacity of liver in rats.

Keywords: Nigella sativa, mitotic activity, liver regeneration, hepatectomy.

INTRODUCTION

Nigella sativa (Family Ranunculaceae) is a widely used medicinal plant all over the world. No adverse effects of nigella sativa has been reported, yet. On the other hand It is reported that N. sativa (0.2 mL/kg) intraperitoneally relieves the deleterious effects of ischemia reperfusion injury on liver. Biochemical parameters like the serum aspartate aminotransferase, alanine aminotransferase lactate dehydrogenase levels and total antioxidant capacity, catalase, total oxidative status, oxidative stress index and myeloperoxidase were determined in hepatic tissue in rats with hepatic ischemia. Results suggested that Nigella sativa treatment protects the rat liver against hepatic ischemia reperfusion injury (1). This study is aimed to evalute effect of Nigella sativa on mitotic activity in partially resected liver, that has not been reported yet.

MATERIAL AND METHODS

Research ethics committee approval was received from the Gazi University. Wistar Albino rats, whose weights were 250–320g and feeding with the laboratory feed, were used. The study included 40 male Wistar albino rats divided into four groups. Group 1: Partially hepatectomyized rats which sacrificed in postop 1st day. Group 2: Partiallly hepatectomyized rats, which was applied oral 1ml/kg Nigella sativa oil after partial hepatectomy and sacrificed in the 1st day. Group 3: Partially hepatectomyized rats which sacrificed in postop 7th day. Group 4: Partially hepatectomyized rats, which was applied oral 1ml/kg/daily Nigella sativa oil after partial hepatectomy and sacrificed in the 7th day. All applicable international, national, and institutional guidelines for the care and use of animals were followed.

One of the rat in group 3 was removed from the study because of exitus at postop 3rd day.

Operations were performed in the first half of the day in order to pretend the effect of daily changing regenerative response. By providing sterile conditions, after abdominal shaving of the rats under the 40 mg/kg Ketamine HCL (Ketas) anaesthesia, laparotomy was performed by midline incision. 70 % hepatectomy was performed by tying liver left lateral and median lobs pedicules with 4/0 silk as Higgins and Anderson defined (2,3). This method was preferred because of the advantages of it that is an easy performing feature and remained liver tissue was non-damaged.

Histopathology and Immunohistochemistry

The resected livers were fixed in 10% neutral buffered formaline solution. The entire liver tissue was sampled and embedded in paraffin. For histopathological evaluation, 5 µm slides were stained with hematoxyline-eosin. Mitotic figure count for 50 high power field were examined by a single pathologist unaware of the group identity.

Immunstaining of Ki67 was performed using the streptavidin-biotin method with a rabbit monoclonal antibody against Ki 67 (clone SP6, Neomarkers, Labvision). Six-µm sections were cut from formaline-fixed, paraffin-embedded tissue specimens, mounted on poly-L-lysine-coated slides, deparaffinized in xylene and washed twice in ethanol. Ki-67 requiredboiling in 10mM Citrate buffer pH 6.0 for 20 minute at microwave oven. Sections were incubated with primary antibody solution for Ki-67 for 1 hour at room temperature. Immunostaining was performed with the streptavidin-biotin complex kit (Dako,Corporation, Copenhagen, Denmark). After incubation, the chromogen specimens were counterstained with Harris hematoxylin and coverslipped. The mitotic count labelled with Ki-67 antibody for 50 high-power-fields was measured for all groups.

Statistical analysis

In the assessment of the data provided in the study, SPSS FOR Windows 16. 0 (SPSS 16. 0 Inc Chicago, IL) statistic packet programs were used. Whether numeric data respect to normal distribution or not was assessed with Shapiro–Wilk test. Cases defined as minimum, middle, and maximum values.

For the inter-group comparison, the Mann-Whitney U analysis and for the paired comparison the Wilcoxon Signed Rank tests were used. In the categorical variables comparison, crosstab statistics were used (Chi-square and Mc Nemar). The limit of statistically significance was determined as (p) 0.05.

RESULTS

There was no statistically significant difference between the weights of the rats in the control groups and study groups. Also, there was no statistically significant difference between the weights of partial hepatectomy material in

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the control groups and study groups (table.1&2).

In the consequence of the comparison made with Bonferroni correction, there was no statistically significantly difference between the weights of the rats on the 1st and 7th day p>0.05). While there was no significant difference between the 1st and 7th days cases of the weight of the hepatectomy materials in the control group (p>0.05), the weight of the cases of 7th day hepatectomy materials statistically was significantly higher than the 1st cases (p<0.05)

Table. 1. Comparison of group 1 and group2

	Group 1 (Control)	Group2	p
Rat weights(g)	283 (250- 320)	284 (250- 320)	>0.05
Partial hepatectomy material weight (g)	7.12 (5.8- 8.4)	7.2 (5.7- 8.6)	>0.05
1st day hepatectomy material weight (g)	3.6 (3.1- 5.3)	4.2 (3.1- 5.5)	>0.05
KI-67 Values(%)	5 (4-8)	10 (5-14)	<0.05*

Table. 2. Comparison of group 3 and group 4

	Group 3 (Control)	Group 4	p
Rat weights(g)	281 (250- 320)	283 (250- 320)	>0.05
Partial hepatectomy material weight (g)	7.1 (5.8- 8.2)	7.2 (5.7- 8.7)	>0.05
7th day hepatectomy material weight (g)	5.8 (3.7- 10.7)	9.9 (5.5- 11.5)**	<0.05*
KI-67 Values(%)	1 (0-2)**	9.7 (6-13)	<0.05*

There was statistically significant difference between Ki67 values of group-2 (figure.2) and group-1 (figure.1) (table.1). Also, There was statistically significant difference between Ki67 values of group-4(figure.4) and group-3 (figure.3) (table.2).

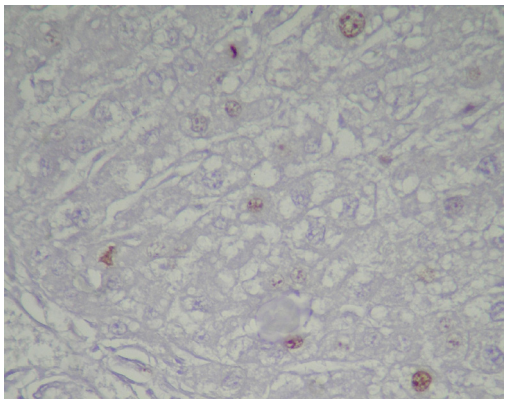

Figure 1) *A sample from group 1. Liver parenchyma showing mild mitotic activity by Ki-67 immunostaining (Ki67 immunostain, X200).*

It was found that the 1st day detected mitosis number of the cases in control group were higher than the 7th day cases (p<0.05). On the other hand there was statistically no significant difference between mitosis number of the cases in study groups (group2 and group4) (p>0.05).

We observed that the ki67 values statistically significantly decreases between 1st and 7th days in control groups. But there was no statistically significant difference between 1st and 7th days in study group (Table 1&2).

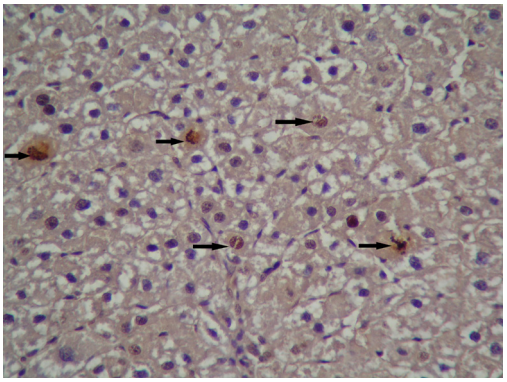

Figure 2) *A sample from group 2. Liver parenchyma showing moderate mitotic activity. Ki-67 positive cells marked by arrows (Ki67 immunostain, X200)..*

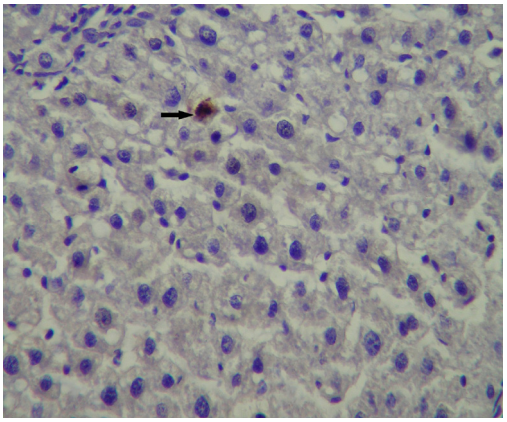

Figure 3) *A sample from group 3. Liver parenchyma showing mild mitotic activity .Ki-67 positive cell marked by arrow (Ki67 immunostain, X200)..*

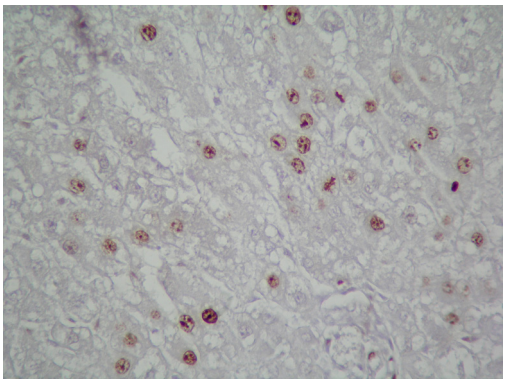

Figure 4) *A sample from group 4. Liver parenchyma showing moderate mitotic activity by Ki-67 immunostaining (Ki67 immunostain, X200)..*

DISCUSSION

Liver has critical metabolic functions regarding all systems. Today, liver surgery can be performed in large centres because of both preoperative run-in and postoperative care and complications. Particularly, liver regeneration, which is the most important event after liver transplantation and better understanding of the factors effects this, will highly influence the models of recruitments (4).

It was shown that, regeneration in the liver tissue after partial hepatectomy begins from first day on (5–7). It was also shown that, functional liver recovering is completed in two weeks after the losing 2/3 of the liver. Active cell replication begins within the first 24 hours after the partial hepatectomy and continues until the organ reaches its initial weight. Within 10 days, there occurs critical regeneration and this case is completed within 4–5 weeks. However, excised lobs don't take the original form. Instead, regeneration occurs with the formation of new lobules and expanding of remained lobules (8). Also, we observed a significant difference between the 1st and 7th days hepatectomy weights of the cases in the control group ($p<0.05$) (Table.2). On the other hand we observed that the ki67 values statistically significantly decreases between 1st and 7th days in control groups. But there was no statistically significant difference between 1st and 7th days in study group. In the light of this we can say daily 1ml/kg nigella sativa oil increase mitotic rate and regenaration capacity of liver in rats.

In the past researches, many markers (DNA syntheses and mitosis number, volume of the liver, cell proliferation and mitochondrial activate) have been used to define the criterions of the liver regeneration (6). Gerdes et al identifi ed the Ki-67 antigen and monoclonal antibody which was composed against the antigen in the cell nucleus (9). Ki-67 protein was identified in all the cell cycle (10). Antigen coverage increase as long as cell cycle keeps proceeding. It reached the highest level in G2-M phase. Monoclonal antibody, which was identified against Ki-67 antigen, has shown in all the phases except G0 phase of cell cycle. We used Ki-67 proliferation index as regeneration mitosis indicator, in our study.

Nigella sativa is an annual flowering plant from Ranunculaceae family, native to southwest Asia which has many food and medicinal uses. Its seeds have pharmacologically active quinone, which possesses several properties such as anti-inflammatory, anti-hyperlipidemic, anti-microbial, anti-cancer, anti-oxidant, anti-diabetic, anti-hypertensive, and wound healing activities. The use of this seeds and oil is common for treatment of many diseases, including digestive diseases, rheumatoid arthritis, asthma, inflammatory diseases, and diabetes. It also has effects on reproductive, digestive, immune and central nervous systems, such as anticonvulsant and analgesic activities (11). Unfortunately, the exact mechanism is not defined clearly. No adverse effects of nigella sativa was reported in human. Also, Nigella sativa oil did not produce any adverse side effects in the doses tested in our study.

It is reported that Nigella sativa treatment significantly decreased pathological changes like focal necrosis and infiltration of leukocytes in ischemia/reperfusion injury in rat liver (1). By this study we can say that nigella sativa oil in 1ml/kg/per day dose increases the mitotic activity and regeneration capacity of liver in partially hepatectomized rats.=

CONCLUSION

In this study, we observed that there was statistically significant difference between Ki67 values of group-2 and group-1. Also, There was statistically significant difference between Ki67 values of group-4 and group-3. The ki67 values statistically significantly decreases between 1st and 7th days in control groups. But there was no statistically significant difference between 1st and 7th days in study group. In the light of this we can say daily 1ml/kg nigella sativa oil increase mitotic rate and regenaration capacity of liver in rats. No adverse effects of nigella sativa was reported in human. Thus, nigella sativa oil can be usable and useful in hepatic disorders. Further clinical studies are necessary.

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