



**RESEARCH ARTICLE**

**Effect of *Ruta Graveolens* Extract on Histopathologic Changes in Mice Livers**

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**ABSTRACT**

Ruta had been used in different nation traditional medicine specifically in Iranian traditional medicine and marked properties had been mentioned for it. In old references using Ruta in some cases caused failure in vital organs of body such as liver and kidney and also mortality. This study had been performed to survey effects of *Ruta graveolens* extract on histopathologic changes in mice livers. This is an experimental study which made on 30 NMRI mice. Mice were divided in 6 quintuple groups. Groups 1 to 4 injected by the doses of 200,300,400 and 500 mg/kg of hydro-alcoholic extracts respectively. Solvent of extract injected in group 5 mice and group 6 mice were intact. The injection was performed intraperitoneal and three times in a week. In the 7th day mice were anesthetized deeply then liver tissues were extracted and after H&E coloring analyzed pathologically. Different pathologic factors were studied in liver of mice. Only common positive factor in all groups were a little inflammation around portal space in comparison with control group. Pathologic results show that inflammation and cell infiltration was increased dose dependent. Any fatty change, fibrosis and evidences of cholestasis were not seen and there was no change in control group subjects. It seems that different doses of hydro-alcoholic extract of *Ruta graveolens* had no toxic effect on histopathology of liver except in high dose. In order to the results high doses (500 mg/kg) of herb can lead to histologic hepatotoxicity and careful care for using this herb should be done in long term with using certain non-toxic doses and avoiding self-medication.

**KEYWORDS**

Ruta, Liver Toxicity, Hydro-Alcoholic Extract

**INTRODUCTION**

*Ruta graveolens* is one of the medicinal plants in Iranian traditional medicine that has a long history in different nations for its remarkable therapeutic properties.

It has more than 85 material parts (from leaves, stems, roots and Seeds) including a variety of glycosides, alkaloids Quinoline, Coumarin, lignin and Flavonoids, using gas Chromatography<sup>2</sup>. Among these substances, flavonoids and in particular rutin (Rutin) has been considered by researchers. Bio- flavonoids can remove free radicals and have a great

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importance. Effects of hydroxyl ethyl Rutine is evaluated in many pharmacological studies<sup>3</sup>. Several clinical and cellular studies have proved strong anticancer effects of this plant, but mainly hematic compounds with the nanogram concentrations have been investigated<sup>4</sup>. It have been shown that *Ruta* in small amounts by countering free oxygen radicals and inhibiting lipid peroxidation of membrane cytotoxic effects on human lymphoma cancer cell and fibroblastoma and also increases the lifespan of animals with cancer<sup>5</sup>. *Ruta*'s hematic compounds in mice can prevent liver cancer carcinogens in mice, but the mechanism of this effect has not been investigated<sup>6</sup>. Aqueous extract of *Ruta graveolens* and its extracted Coumarin have the ability to inhibit potassium channels of myelinated nerves which can be useful in the treatment of nervous system diseases such as multiple sclerosis (MS)<sup>7</sup>. Because of the nature of the plant is warm and dry in the third degree and it is basically a purely medicinal plants and according to the strength and breadth of the effect of the plant, the side effect is predictable. Especially in people who have hot and dry-tempered bilious temperament so-called in traditional medicine, this plant can cause further complications<sup>8,9</sup>. Considering that the liver tissue metabolizes a large number of internal and external constituents, and this tissue plays an important role in detoxification of the body, In this study the effects of different doses of extract of *Ruta graveolens* on liver tissue of mice evaluated histopathology.

## MATERIALS AND METHOD

In this experimental study, 30 male NMRI mice in the weight range of 25 to 35 gram were used. Mice were randomly divided into 6 groups of 5 animals. Except in test time, the mice had access to food and water freely. Diet of mice was including prepared food (Pars animal feed factory) and cleaned up water. They had 12 hours dark cycle and 12 hours of light cycle. The animals were kept in group and in the experiment day (at least one hour before the start of the experiment), the group that had

already been marked were placed in separate cages. Laboratory temperature was kept in range of  $22 \pm 2$  °C, and each mouse was used in only one trial. All of principles of research ethics in animals was performed standardly<sup>10</sup>.

Aerial parts of *Ruta graveolens* in May were collected and identified in Rasht and confirmed by the expert in faculty of Agriculture in university of Guilan. After drying, the plant was crushed by the mill. 40 grams of dried plants in a flask with 250 ml of water and 250 ml of 70% ethanol were mixed, were heated in temperature of 40 °C for half an hour. After placing the sample at room temperature for 24 hours, it was filtered to remove plant pulp from plant. Solution containing active ingredients transferred into a volume balloon of vacuum distillation unit (Rotary evaporator) and after evaporation of alcohol and water, the resulting solution was transferred to the oven with 50 °C 11 to dry completely. Weight of dry extracts obtained 8 grams.

Mice were injected in groups 1 to 4, respectively, at doses of 200, 300, 400 and 500 mg/kg of extract and group 5 (control) was injected by solvent extraction (normal saline) for a week and every other day intraperitoneally and group 6 remained without any injection. At the end of the seventh day the rats were anesthetized with a mixture of Ketamine and Xylazine deeply and the liver tissue of them were kept in 10% formalin and then stained with Hematoxylin & Eosin was analyzed pathologically.

## RESULTS

In microscopic examination of tissue sections prepared from the liver of experimental and control groups, various changes were observed and evaluated by index of inflammatory markers in the portal space, existence of Biliary pigment in the port space, inflammation on parenchymal tissue and regenerative changes in the hepatocytes. In each group, 5 sections of tissue were prepared and the mean changes in tissue were shown separately for each group in Table 1.

Table 1: Histopathological changes in the liver tissue mice with different doses of the extract *Ruta Graveolens*

Groups	Inflammation of portal space	Biliary pigment in portal space	Parenchymal inflammation	Regenerative changes in hepatocytes
Dose 200 mg/kg	Mild	-	-	-
Dose 300 mg/kg	Mild	1+	Mild	Scattered
Dose 400 mg/kg	1+	1+	-	Scattered
Dose 500 mg/kg	1+	Mild	Mild	1+ infiltration containing a large number of nest
Control (Normal saline injection)	1+	-	-	-
Sham (no injection)	Very mild	-	-	-

In the first group (200 mg/kg) normal structure of liver lobules was preserved. Central lobule vein was normal; the sinusoidal wall was distinct and endothelial lining in a row was seen in the wall. Mild inflammatory cells of the lymphocyte type were seen in portal space. Nucleus of hepatocytes had sharp border and nucleus/cytoplasm ratio was normal (Figure 1).

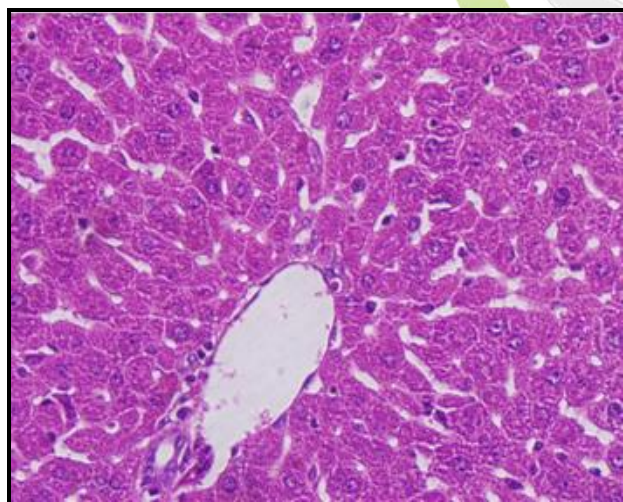


Figure 1: Microscope image of the liver tissue in the first group (200mg/kg of *Ruta graveolens* extract) (Magnifying  $\times 400$ )

In the second group (300 mg/kg) normal structure of liver lobules was preserved. Central lobule vein was normal; the sinusoidal wall was distinct and endothelial lining in a row was seen

in the wall. Mild inflammatory cells of the lymphocyte type were seen in portal space. Regenerative changes and inflammation of parenchymal tissue of liver were seen diffusely in hepatocytes (Figure 2).

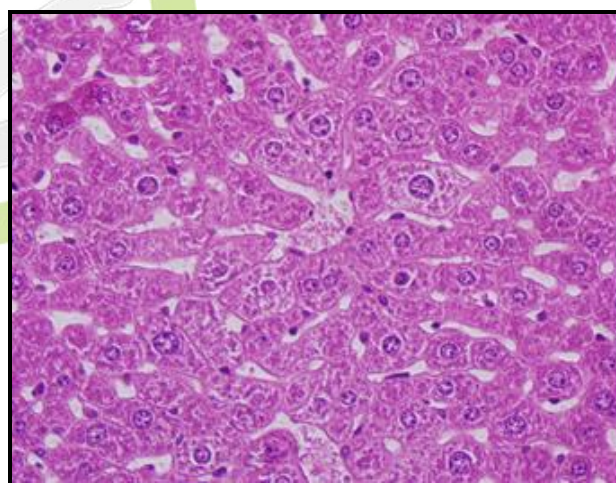


Figure 2 - Microscopic image of the the liver tissue in the first group (300mg/kg of *Ruta graveolens* extract) (Magnifying  $\times 400$ )

The third group (400 mg/kg) had no changes in normal structure of liver lobule. Central lobule vein and sinusoidal wall were distinct and floored by a layer of endothelial cells. Portal space was containing mild inflammatory cells in type of lymphocyte and biliary pigments. Regenerative changes were seen in hepatocytes diffusely (Figure 3).

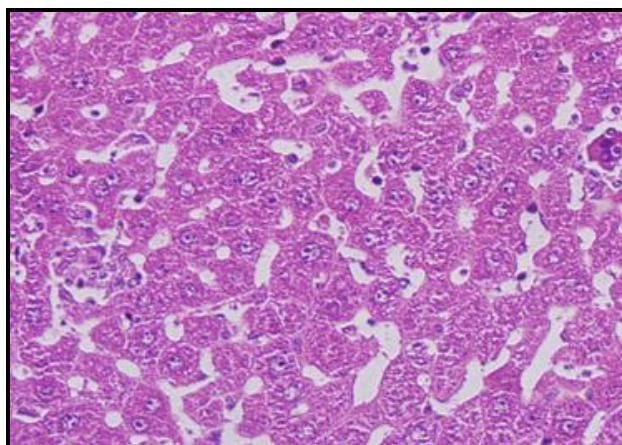


Figure 3 - Microscopic image of the the liver tissue in the first group (400mg/kg of *Ruta graveolens* extract) (Magnifying  $\times 400$ )

The fourth group (500 mg/kg) also had no change in normal structure of liver lobules. Central lobule veins and sinusoidal wall were floored by a layer of endothelial cells. Portal space was containing inflammatory cells of lymphocyte and neutrophil and biliary pigment. Regenerative changes and a lot of nest were seen in hepatocytes (Figure 4).

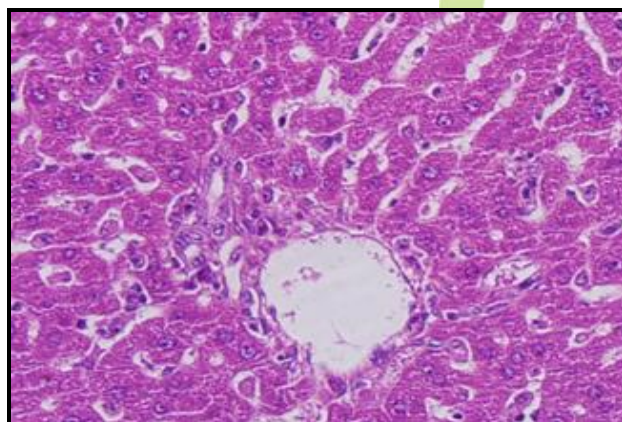


Figure 4: Microscopic image of the the liver tissue in the first group (500mg/kg of *Ruta graveolens* extract) (Magnifying  $\times 400$ )

In control group (with normal saline injection) and sham group (with no injection) the normal structure of the liver lobules were preserved. Central vein of lobules was normal. Sinusoidal wall was distinct and have a layer of endothelial cells. Portal space had a mild degree of inflammatory cells of lymphocyte. Hepatocyte's border was well-defined and nucleus/cytoplasm ratio was normal (Figure 5).

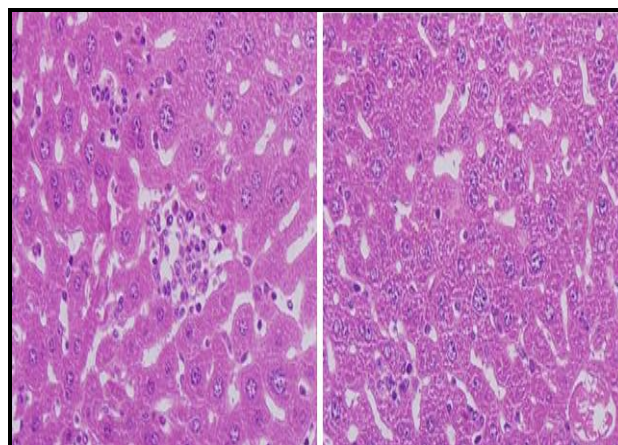


Figure 5: Microscope image of the sham and control groups (magnifying 400 $\times$ )

## DISCUSSION

This histopathologic study showed that the group of mice received 200 mg/kg *Ruta graveolens* extract had mild lesions in the liver were not clearly separable from the control group. But the group received 500 mg/kg of extract in comparison with those who received lower doses of *Ruta graveolens* extract and control and sham groups had significant inflammatory and structural changes. There were mild and scattered inflammatory changes in the cases of 300 mg/kg and 400 mg/kg concentrations. As a simple conclusion these changes can be contributed to effects of high dose of *Ruta graveolens* that causes destruction of liver tissue. As it had been mentioned in traditional medicine resources, high consumption of *Ruta graveolens* can lead to poisoning and overusing it causes inflammation and distention of the digestive tract, low body temperature, weakness of pulse, increased urination and swelling of the mucus of the uterus<sup>8</sup>.

*Ruta graveolens* had a variety of compounds that some of them are Coumarin, alkaloids, flavonoids and essential oils and essences<sup>11</sup>. Methoxsalen, Quercetin and flavonoid compounds are compounds of its extract. According to reports, these compounds can be effective on cellular DNA, inhibit the DNA replication and cell proliferation and stimulate cell apoptosis<sup>13-15</sup>. Also Xanthotoxin of this plant can cause destruction and tissue damage<sup>16</sup>.

Also Psoralens in the *Ruta graveolens* extract prevent the replication and proliferation of DNA and mitosis<sup>17</sup>. In Agraa and et al study they showed that apoptosis of hepatocytes and renal tubular cells were occurred in gouts treated with the extract of Ruta<sup>18</sup>. Also in another study, cell death and apoptosis in colonic crypts were occurred in mice treated with Quercetin and Rutine prepared from *Ruta graveolens*<sup>19,20</sup>. In Freire and colleagues study similar to our study, had shown mild to moderate hepatotoxicity in laboratory findings using hydroalcoholic extract of Ruta in mice infected with *Vampirolepis nana*. Although dried *Ruta graveolens* is known as a plant with few complications commonly, they observed that the aqueous extract of it has a more detrimental effect than the ethanolic extract<sup>21</sup>.

## CONCLUSION

The results of the present study showed that the *Ruta graveolens* contain active ingredients that in spite of remedial results, in high doses (500 mg/kg) can lead to histologic hepatotoxicity. These results are in contrast with the general thinking of lack of complications in herbal medicine such as *Ruta graveolens*. Careful care for using this herb should be done in long term with using certain non-toxic doses and avoiding self-medication.

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