RESEARCH ARTICLE

Hepatoprotective Effect of *Lallemantia Royleana* Seeds Extract Against Rifadin Toxicity in Male Albino Mice

Mohammed A. Jawad,[1] Abed J. Kadhim,[1] Saif Y. Hasan[2]

[1]*Al-Nisour University College, Baghdad, Iraq*
[2]*National University of Science And Technology, Nasiriyah, Iraq*

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ABSTRACT

The study aimed to highlight the protective effect of *Lallemantia royleana* seed extract (Balangu) in protecting the liver against the toxicity of Rifadin. The study was conducted on the male Swiss albino mice (40 mice), aged 5 to 8 weeks and weighed 25 to 30 g, which were divided into four animal groups: the first group orally administrated (0.1 mL) of saline solution (0.9%) for 28 days to represent the control group, second group administrated with 0.1 mL of Rifadine (1.5 mg/kg/day) for 28 days. The third group was administered (0.1 mL) of alcohol extract of Balangu seeds (1%) for 28 days. The fourth group was administrated with seed extract and Rifadin for 33 days. Histopathological changes in the liver tissue of the experimental groups were reported as a loss of the radial arrangement of the hepatic cords in the central lobular region and in the surrounding areas with moderate/severe congestion in the drug-treated group, the seed and drug extract group, the retention of hepatic cords were observed in the radial regulation, especially in the area around the central vein with moderate blood congestion (Mild/ Moderate Congestion) in the central veins, and the presence of many numbers of Balone-Shape cells and Hepatic Vacuolated cells. The histological composition of the Balangu seed extract group was similar to that of the control group, but there is infiltration of mononuclear leukocytes in the central lobular region.

Keywords: Extract, *Lallemantia royleana*, Rifadin, Toxicity

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INTRODUCTION

The liver plays a key role in the biological transformation of drugs and toxins, which are the main cause of several symptoms observed in the liver. There are more than 600 drugs that cause liver damage, which can be repaired if they are stopped. However, the condition can become worse if they continue to be used.[1]

Some drugs also cause liver damage more than other drugs. Some people are more sensitive to the drug that causes hepatic damage than others who take the same drug and the same dose.[2] This condition is called liver injury caused by Drugs, Drug-Induced Liver Injury (DILI)), a major health problem in the world resulting from the increased exposure to several drugs and modern medicines consumed by prescription or alternative medicine such as supplemental medicines and supplements. The liver damage by various drugs leads to the emergence of different clinical patterns, including fatty hepatitis, Steatohepatitis, inflammation Hepatitis, chronic hepatitis and cirrhosis. Also, it can cause vascular damage, which reflects the susceptibility of drugs to cell-induced liver damage through mechanisms and pathways.[3] Isoniazid, Rifampicin, and Ethambutol toxic effects in the liver.[4]

Several studies have indicated the role of some plant extracts in the protection of hepatic cells. *Lallemantia royleana* (Balangu) belongs to the Labiatae family and is present in many countries in Europe and Asia, such as Turkey, Iran, India,[5] Uzbekistan, and Kyrgyzstan,[6] a leafy plant covered with dense foliage. The stalk of the plant is simple or branchy, with a length of 30–50 cm. The bottom leaves of the plant are oval and hugged with edges that are oppressed. The seeds are smooth and dark brown, 2.5 cm long. -3 mm[6] and diameter 0.580–0.875 mm. The seeds of the Balangu plant contain chemicals, including proteins by 26.60%, oil by 18.27%, and fiber 30.67%, as a good source of these materials.[5] The seed oil is bright green and contains a variety of fatty acids, including linolenic acid (26.1%), oleic acid (59.4%), Palmitic acid (10.1%), stearic acid (3.2%), and sitosterol (0.28%).[7]

The therapeutic applications of Balangu are the use of *L. royleana* (Balangu) extract in alternative medicine and the treatment of stomach diseases and neurodegenerative diseases,[6]

**Author for Correspondence: mohammed.a.medical.lab@nuc.edu.iq*

a painkiller, and a sedative agent for urinary tract problems and cough. The seeds are used to treat cysts, bruises, and infections,[8] as pointed out by Mahmood *et al*.[9] The alcohol extracts of *L. royleana* (*Pseudomonas aeruginosa, Enterobacter cloacae*, *Escherichia coli, Staphylococcus aureus*) can cause skin diseases and gastrointestinal problems. The gels were extracted from the Balangu seeds at a concentration of 0.01 g. The anesthetic's duration and strength of gel are similar to the gelatin 2% industrial lidocaine,[10] and Balangu seeds harm Cholesterol and triglyceride levels in serum.[11]

This study aimed to reduce the toxic effect of Rifadin in the liver by using the alcohol extract of the Balangu seed. To evaluate this effect through histological studies to know the tissue changes on the liver sections after treatment with the Rifadin and seeds extract of the Balangu.

MATERIALS AND METHODS

The experiment was conducted in the laboratories of the Department of Biology, College of Education for Pure Sciences (Ibn al-Haytham), University of Baghdad, Iraq for the period (20-10-2014) until (20-6-2015), where the study was conducted on the swiss albino male mice (40 mice). They ranged in age from 5-8 weeks and weighed 25–30 g.

The animals were divided into four groups and at the rate of (10) mice for each group as follows:

- *Group 1 (control group):* Mice in this group orally dosed 0.1 mL of normal saline solution with a concentration of 0.9% for each variant daily for 28 days.
- *Group II (group treated with the Rifadin drug)*: Mice were given in this group 0.1 mL of Rifadin at a concentration of 1.5 mg/kg/day for 28 days.
- *Group III (Group treated with the extract of the seeds of the Balangu):* Mice in this group administrated with 0.1 mL of alcohol extract of Balangu seeds 1% (w/v). The duration of the dosage was 28 days as well.
- *Group IV (group treated with the Rifadin drug and extract of the seeds of the Balangu):* Animals were given 0.1 mL of the Balangu extract (1%) for five days and treated with 0.1 mL with the extract of Balangu and Rifadin (0.1 mg/kg/day) for a period of 28 days, bringing the total number of dosage days for this group to 33 days.

The mice were killed by spinal dislocation then their livers were collected for testing.

According to Bancroft *et al*.,[12] histological preparation was used in tissue preparations to examine the histopathological changes.

RESULTS

Microscopical examination of liver tissue in mice which administered Rifadin drug (1.5 mg/Kg/day) shows that there was a complete loss of the natural liver cell structure, which affect in turn the hepatic cords of the centrilobular region, and it is a rounding area compared to the control (Figure 1A). Decomposition of Liver tissue and liver cell necrosis was found in large numbers within the Centrolobular region (Figures 1B and C). The necrotic cells appear as colored, swelling, and

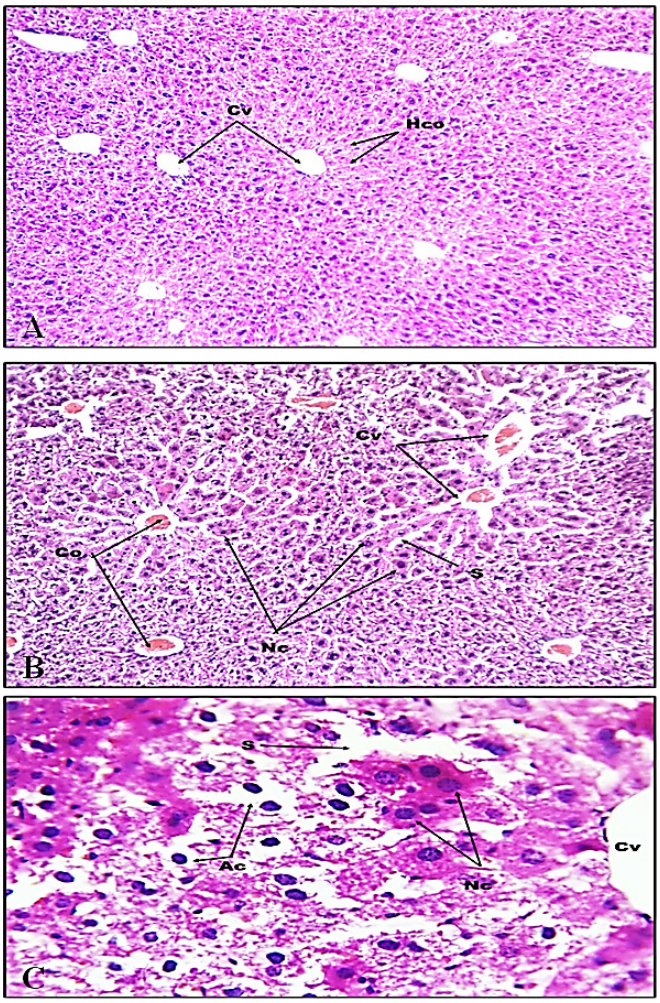

Figure 1: Cross-sections in the liver of male albino mice A) control group showed normal architecture in the liver tissue 10x, B) Rifadin treated group showed the looses in the liver tissue and the congestion in central veins 10x, C) Rifadin treated group notice necrotic cells and apoptosis 40x.cv- central vein, Hco-hepatic cords, co-congestion-sinusoids, Nc-necrotic cells, Ac-apoptotic cells (H & E).

increased number of apoptotic cells (Figure 1C). The central vein area shows congestion varied from severe to moderate with increased hepatic sinusoids (Figure 1B).

There were many vacuolated, balone-shaped, and karyomegaly cells near the hepatic lobules with increasing hepatic binuclear cells. In addition to the presence of a small number of mononuclear leukocytes in the centrilobular region, such changes were not seen compared to the control tissue (Figure 2A).

The hepatic tissue structure in the mice of the group which administered alcoholic extract of *L. royleana* seeds (1%) showed mild changes compared to control (Figure 2B). Mild congestion around the central vein and mononuclear leukocytes (small number) were seen near the centrilobular region and portal area, with some inflammatory cells resulting from the formation of the focal inflammatory region (Figure 2C). Such changes were not seen in the hepatic tissue of the control group (Figure 1A). Vacuolated hepatic cells and karyomegaly

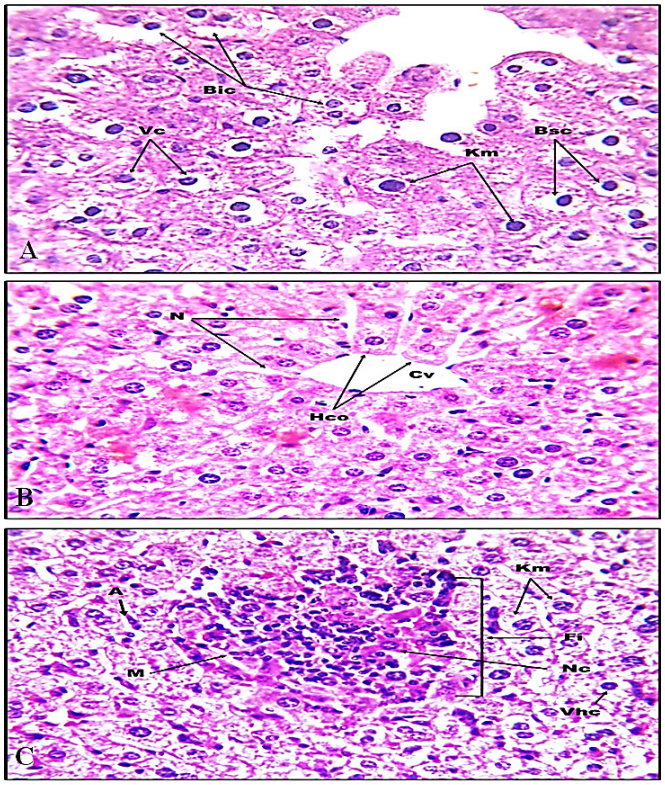

Figure 2: Cross-sections in the liver of male albino mice A) control group showed normal architecture in the liver tissue 10x, B) Rifadin treated group showed the looses in the liver tissue and the congestion in central veins 10x, C) Rifadin treated group notice necrotic cells and apoptosis 40x.cv- central vein, Hco-hepatic cords, co-congestion-sinusoids, Nc-necrotic cells, Ac-apoptotic cells (H & E).

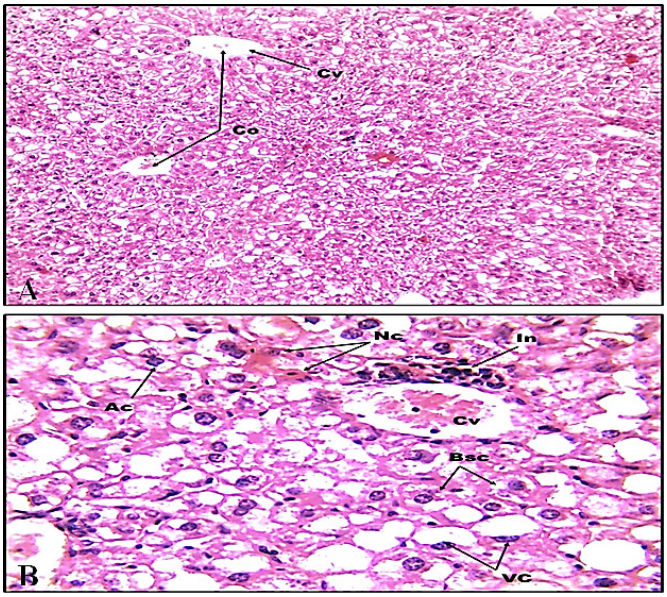

Figure 3: Cross-sections in male albino mice treated with alcoholic extract of *L. royleana* seeds (1%) and Rifadin (1.5 mg/Kg/day) showed: A-mild congestion10x, B-mild infiltration, decreasing the number of necrotic and apoptotic cells 40x. Co-congestion, In-infiltration, Nc-necrotic cells, Ac-apoptotic cells (H&E).

cells were also seen near the hepatic lobules, in addition to a small number of necrotic and pre necrotic cells were also seen compared to Rifadin administrated mice group (Figure 2C).

The hepatic tissue of mice administrated alcoholic extract of *L. royleana* seeds (1%), and Rifadin drug appeared as a relatively normal structure (normal hepatic cord and tissue), especially around the central vein area with mild to moderate congestion (Figure 3A). In addition, many other cells like; balone-shaped cells and vacuolated hepatic cells were seen in the centrilobular region and portal area, which give the tissue an appearance of vacuolated tissue compared to control (Figure 3B).

DISCUSSION

Histopathological examination of Rifadin drug administered hepatic mice showed a loss of normal hepatic architecture of hepatic cords available in the centrilobular region compared to the control group. Such results agreed with old[13,14] studies that reported that Rifadin's administration might lead to loss of normal hepatic architecture affect the hepatocytes and hepatic lobules). The results also reported that a large number of necrotic and apoptotic cells were present in hepatic tissue available within the centrilobular region and portal area. Such a result was similar to Gond and Khadabadi.[15] They reported that using Rifadin drug may lead to hepatic histopathological changes (rupture of the plasma membrane with subsequent hepatocytes death). Sharma and Mohan.[16] also investigated the effect of Rifadin as an anti-tuberculosis drug, and their study showed that such treatment might stimulate cell apoptosis through stimulation of Cytochrome P 450 and Cytochrome C enzyme, in addition, to increase in lipid peroxidase enzyme activity which destroys the hepatic membrane phospholipids and other structure through stimulation of enzyme oxidation process.

Stimulation of enzyme lipid peroxidation process may change the permeability of hepatic cells, which in turn release enzyme Cytochrome C that stimulates the hepatic cellular apoptotic process, meanwhile stimulation of Endonucleases and Caspases as a result of an increase in Ca^{++} concentration may increase DNA destruction and cellular apoptosis.[17,18]

This study also showed that many hepatic Vacuolated cells were present in the centrilobular region due to lipid accumulation and reduction in the B-oxidation process with the decrease in free radical and ATP levels.[19,20] The reduction in ATP level is one of the factors that affect the appearance of the vascular steatosis and the deacylation-reacylation reaction process.[17]

The microscopical examination of hepatic tissue of male mice administrated alcoholic extract of *L. royleana* seeds (1%) showed mild changes (relative normal tissue architecture) compared to control. This effect may be related to the presence of Linolenic acid (Omega 3), Flavonoids, Alkaloids, Tannin, and polyphenol in the plant extract of *L. royleana* acting as anti-oxidants and protect hepatic cells from the effect of free radical and other oxidizing agents. Such results were agreed with Hamzawy *et al*.,[21] who reported that alcoholic extract of *Thymus vulgaris* has a great role in keeping normal hepatic

tissue architecture (around the central and portal vein). This effect was related to the presence of phenolic compounds (Thymol and Carvacrol), which have anti-oxidant action. On the other hand, this study showed the presence of mild congestion in the central hepatic vein with the appearance of Mononuclear leukocytes in the centrilobular region and portal area with mild aggregation of inflammatory cells within hepatic lobules due to the formation of focal inflammation compared to normal hepatic tissue (control). Vacuolated hepatic cells were also seen in the hepatic lobules. Such effect can be explained by the presence of Palmitic acid and Oleic acid. The effect of Palmitic acid was mentioned in previous researches,[22] who reported that Palmitic acid affects hepatic cells and plays an important role in the stimulation of hepatic cell inflammation. At the same time, the effect of Oleic acid was investigated by Cui *et al*.,[23] who showed that hepatic cells (HePG2) exposure to a high concentration of Palmitic acid may stimulate the release of interleukin-8 (IL-8). Moreover, when exposed to Oleic acid, it may stimulate the release of TNF-α, which attract T- lymphocytes and neutrophils through binding to the endothelial intercellular adhesion molecule-1 (ICAM-1), followed by the release of free radicals, which trigger the formation of hepatic steatosis which explained the presence of some hepatic vacuolated cells in the lobular region.

This study demonstrated relative normal hepatic tissue and hepatic cord with mild to moderate central vein congestion and a few necrotic cells compared to the other group that administered refadin drugs alone. Such results may be related to the presence of Linolenic acid (Omega-3), Flavonoids, and phenol (in the *L. royleana* seeds extract) which act as anti-oxidant and stimulate glutathione enzyme, which plays an important role in Rifadin metabolism, decreasing drug oxidation effects and remove the toxic action of the free-radical which in turn provide hepatic protection against Rifadin drugs.[24]

The action of plants extracts has the main role in hepatoprotection because of their anti-oxidant properties. It is noticed that these plants are rich in active components such as coumarins, glycosides, flavonoids, alkaloids, and tannins that are well known as liver protective components.[25]

CONCLUSION

From the above results, the study reported that *L. royleana* seeds extract, when administered to male mice, provides partial protective effects to hepatic tissue from the toxic Rifadin drug effects. This action may be related to the active constituent of the plant extract, which gives the protection effect, and at the same time, some of them may produce mild histopathological changes may be related to a high concentration of plant seed extract given to male mice.

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