Hepatoprotective Effect of Lallemantia royleana Seeds Extract Against the Toxicity of Rifadin Through Reduction BAX Expression in Liver Tissue of Albino Mice

*Mohammad Fahdil Abdulhussein, **Zainab Thamer Showait Al-Asady
*Baghdad University/ College of Education for Pure Science
**Al-Karkh University of Science/College of Science/ Department of Microbiology

Abstract. This study was aimed to investigate the role of crude alcoholic extract of Lallemantia royleana seeds through reduction the hepatotoxicity of rifadin drug in liver tissue. The animal (40 mice) were divided into four groups the first group treated with normal saline (0.9%) for 28 days as a control, the second group treated with rifadin (1.5 mg/kg/day) for 28 days, third group treated with alcoholic extract of Lallemantia royleana seeds (1% w/v) for 28 days, while the forth group treated with alcoholic extract of seeds alone for 5 days and with alcoholic extract and rifadin for 28 days, so the total period of this group is 33 days. The liver specimens were collected and processed with immunohistochemical staining kit to detect Bax protein in hepatocytes. The microscopic examination results of liver slides coloured with immunohistochemical staining technique in animal experimental groups showed increased in the number of cells with positive immunoreaction for Bax in animal group treated with rifadin drug comparing with other experimental groups (seeds extract and drug group, seeds extract group, control group) which showed significant decreasing in the number of cells with positive immunoreaction for Bax.

Keywords. Protective effect, Hepatotoxicity Lallemantia royleana, Rifadin

1. Introduction
The liver play essential role in biotransformation of drugs and toxins were considered the main cause for many symptoms that may noticed in the liver [1] and play important role in food digestion process by secreting the bile and it produce, storage and release the carbohydrate, lipids and cholesterol and it storage vitamins A1, B12, B9, D and iron and produce many of important enzymes, hormones and coagulation factors. The liver also play important role in detoxification and defense the body from different agent like alcohol, drugs and heavy materials [2].

There are more than 600 drugs cause liver injury which used to treat different diseases, the situation could become worse in the case of the continued use of such drugs [1], and some drugs cause liver damage more than other drugs also, some people are allergic for the drug that causes liver damage compared to other people taking the same drug and the same dose [3], this condition is called drug induced liver injury (DILI) that are the major health problem in the world resulting from increased exposure to a number of modern drugs that is consumed by medical prescription or non-medical.
prescription such as complementary drugs and dietary supplements [4], and the rifadin drug is a bacterial antibiotic used to treat tuberculosis resulting from Mycobacterium tuberculosis, where the drug is derived from the rifamycin antibacterial group, the drug works through inhibition the formation of RNA in Mycobacterium tuberculosis and other types of bacteria [5], this drug therapy for patients with tuberculosis has a period of up to six months, which is the minimum time that antibiotics need to eliminate all the bacteria present in the lungs and lymph nodes, so the drug is used usually with group as antibiotics like isoniazid, pyrazinamide, ethambutol, streptomycin [6].

Many studies have pointed the role of alternative medicine in protect hepatocytes, and used plant extract as anti-bacterial, anti-toxin, for vomiting, inflammation and treatment the liver disorders [7]. Lallemantia royleana (common name is Balanco) is plant from a family Labiatae exists in many countries in Europe and Asia, especially Turkey, Iran and India [8] Uzbekistan and Kyrgyzstan [9], and the features of plant seeds oil extract is a clear, green in color and contains the active substances, which is a group of fatty acids (10). Lallemantia royleana seeds extract used in alternative medicine in the treatment of the stomach diseases, nervous debility [9], analgesic and sedatives factor to the problems of the urinary system and cough, the seeds are used for the treatment of abscesses and inflammations [11] . Mahmood et., al. (2013) [12] referred that the alcoholic extract of Lallemantia royleana seeds have inhibitory effect of bacterial strains (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterobacter cloacae) that cause skin disease and Gastrointestinal system problems, on the other hand Atabaki & Ezatti (2014) [13] clarified that the gel material were extracted from Lallemantia royleana seeds in 0.01 gm/ml it has analgesic effect and that the duration and strength of anesthesia by the gel be similar to lidocaine 2% that prepared artificially.

Due to importance of the liver, so it has used several techniques to evaluate its effectiveness, including the immunohistochemistry (IHC) [14] and it is technique where detect biomarker in the tissue mediated by antibodies [15], so this technique has used in recent years, mainly for the diagnosis of pathological infections that infect the human liver and then expand its use to include pathological and toxicity experimental studies, also it used to distinguish the types of cells, receptors expressive on cell surfaces, cytokines and chemical produced by cells, as well as changes in the cells because it functional effectiveness, such as cell stimulated to produce and release enzymes [14]. Therefore, the aim of this study is to test the effect of Lallemantia royleana seeds extract through protect the liver from rifadin drug toxicity by using IHC technique to detect the expression of BAX as proapoptic protein in albino mice.

2. Materials and Methods

2.1. Preparation as Alcoholic Extract of Lallemantia royleana Seeds

The plant seeds have been obtained from the local market, after a cleaning the seeds from the impurities, they were grinded using electric grinder until obtained a fine powder. 50 gm of seeds powder have been taken and extracted by sexulate apparatus by 500 ml methanol 95%, and the extraction operation continued for 7 hours at 60°C and leaved the final extract in water bath at 37°C for three days to allow the evaporation of alcohol from it. The crude extract has been obtained with oily textures and clear green color 1% weight/volume and the extract storage in dark bottle at 4°C until used [16].

2.2. Experiment Design and Experimental Animals

The males of Mus masculus have been obtained from national centre for health supervision and pharmaceutical research, the animals have been bred in animal house in biology department- college of Education for pure sciences (Ibn al-haitham)-university of Baghdad, the animals placed under laboratory conditions (temperature between 4+26°C and light: dark cycle 12:12 hour), and were gave pellet and water continuously. The mice were divided into four groups (10 animals for each group):

- **Control group**: the animals of this group treated orally with 0.1 ml of normal saline (0.9%) for 28 days.
- **Rifadin drug treated group**: the animals of this group treated orally 0.1 ml of rifadin drug (1.5 mg/kg/day) for 28 days.
- **Alcoholic extract of Lallemantia royleana seeds treated group**: the animals of this group treated 0.1 ml of alcoholic extract of plant seeds (1% w/v) for 28 days.
- **Alcoholic extract of plant seeds and rifadin drug treated group**: the animals of this group treated orally 0.1 ml of alcoholic extract of plant seeds (1% w/v) for 5 days, and with alcoholic extract (1% w/v) and rifadin (1.5 mg/kg/day) together for 28 days, so the total treated period of this group was 33 day.

### 2.3. Immunohistochemical Staining Method

The kit that detect Bax protein was used and manufactured by the company of SANTA CRUZ BIOTECHNOLOGY, INC.USA, and the liver samples has been processed according to Taylor and Rudbeck(2013) (15) the histological slides were checked by using light microscop, and 1000 cells Assayed to get apoptotic index through the following equation [17]:

\[
\text{Apoptotic Index} (%) = \frac{\text{Number of marked cells with Bax protein}}{\text{Number of total cells (marked + unmarked)}} \times 100
\]

### 3. Results and Discussions

The results of microscopic examination for liver slides that colored with IHC technique to detect Bax protein expression in the cytoplasm of hepatocyte of experimental animal groups and statistical analysis for apoptotic index (figure 1) showed there was a significant increase (P<0.05) in percentage of cells that showed positive immunoreaction for Bax protein in animal group treated with rifadin drug (1.5 mg/kg/day) (figure 2) comparing with other animal groups (seed extract and drug, seed extract, control) (figure 3,4,5). This refers to the role of rifadin drug in stimulating apoptosis in hepatocytes, and the apoptosis was occurred because the drug stimulating the cytochrome P450, which results in an increase in the production of toxic metabolites, as well as the drug works on depletation of glutathion (GSH) that works as antioxidants and removing oxidant factors from cells [18], and cell interference in the process of apoptosis as a result of oxidative stress, the resultant formation of free radicals and mitochondrial membrane rupture release cytochrome-C and activating Caspase-3 pathways, so that cause DNA strands cutting and damage [19], this result associated with Significant elevation ALT, AST levels in the serum of mice that treated with rifadin drug(20 ) The results showed significant decrease (P<0.05) in the percentage of cells that showed Positive immunoreaction for Bax protein in animal groups treated with Lallemantia royleana seeds extract , plant extract and rifadin drug together comparing with rifadin drug treated group, and this result of apoptotic index was came compatible with microscopic examination results in decrease of positive immunoreaction for Bax protein in this animals group.

The causes of reduction positive immunoreaction for Bax protein is presence of active compounds in Lallemantia royleana seeds extract representative by linolinic acid (Omega3) Flavenoids, multiple phenols, alkaloids and tamin(10), which are antioxidants, that play an important role in removing oxidative stress, and then reduction the number of apoptotic cells and this refer the protective effect of plant seeds extract in protect the hepatocyte from oxidative stress and from entering into apoptosis. In the other hand the results showed there was a significant increase (P<0.05) in percentage of cells that showed positive immunoreaction for Bax protein in the liver of animals group treated with plant seeds extract and drug together comparing with seed extract and control groups, so this increase is due to rifadin drug effects in stimulating incidence of apoptosis in hepatocytes [18], but the treatment of at plants seeds extract have reduced the effect of the drug, in the other side there was no significant differences (P>0.05) noted in percentage of cells that showed positive immunoreaction for Bax protein withen groups treated with plant seeds extract and control group, these results was agreed with El-Ghonaimy(2015) [21], in his study on effect of ginger Zingiber officinale on metalaxyl hepatotoxicity as an anti-fungal, he was noted significant increase in Bax expression ratio in animals treated with drug comparing with group treated with ginger and metalaxyl and control group, he was indicated significant
decrease in Bax protein expression ratio in group treated ginger and metalaxyl comparing with group treated with metalaxyl.

The results of current study was agreed with Ibrahim et al. (2015)[22] in his study on the effect of the aqueous extract of Camellia sinensis (Green tea) leaves against the hepatotoxicity from copper nanoparticle, so he was noted significant increase in Bax protein expression in animals treated with copper nanoparticle comparing with other groups (aqueous extract of green tea and copper nanoparticle, aqueous extract, control) and in contrast, he was noted significant decrease in Bax protein expression in aqueous extract of green tea group and in extract and copper nanoparticle together comparing with copper nanoparticle treated group and he showed there was not significant differences between extract group and control. On the other hand there is investigation mentioned that both manuka and tulh honeys have a protective effect against CISP-induced liver and kidney toxicity as demonstrated by decreasing liver and kidney function, the mechanism was to limit the expression of BAX and caspase-3 as apoptotic signals (23).

Thus, we conclude that the alcoholic extract of Balanco seeds provided partial protection to the Hepatocytes due to its active antioxidants.

![Figure 1. Apoptotic index value in mice groups treated with rifadin, plant seeds extract only and rifadin and extract together as well as control (Values are mean + S.E) at significant level (P<0.05).](image)
Figure 2. Section in mice liver treated for 28 days with rifadin (1.5 mg/kg/day) showed increase in number of hepatocyte with positive immunoreaction for Bax protein (200 X). → area colored with Dap chromagen.

Figure 3. Section in mice liver treated for 28 days with rifadin (1.5 mg/kg/day) and plant seeds extract (1% w/v) showed decrease in number of hepatocyte with positive immunoreaction for Bax protein (200 X). → area colored with Dap chromagen.
Figure 4. Section in mice liver treated for 28 days with plant seeds extract (1% w/v) showed decrease in number of hepatocyte with positive immunoreaction for Bax protein (200 X). → area colored with Dap chromagen.

Figure 5. Section in mice liver as a control showed decrease in number of hepatocyte with positive immunoreaction for Bax protein (200 X). → area colored with Dap chromagen.

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