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Original article

THE EFFECT OF *LALLEMANTIA ROYLEANA* SEEDS EXTRACT IN MODIFYING THE INFLAMMATORY RESPONSE AND BAX PROTEIN EXPRESSION IN THE KIDNEY TISSUE OF ALBINO MICE THAT TREATED WITH RIFADIN

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Abstract

Background. It has become known that the repeated use of therapeutic drugs for some medical conditions is accompanied by the appearance of side effects from these drugs, so many have resorted to using alternative medicine to treat some diseases or to reduce the symptoms of the disease due to the effective substances that herbs and medicinal plants contain.

Purpose. The aim of this study is to examine the effects of *Lallemantia royleana* (Balangu) seed extract in modifying the inflammatory response and BAX protein expression as a marker of cell death after treatment with Rifadin.

Materials and methods. Twenty-four Swiss albino mice, 5 weeks old, were divided into four groups: 1) control group treated with normal saline, 2) group treated with 1.5 mg/kg/day Rifadin, 3) group treated with *Lallemantia royleana* (Balangu) seed extract, 4) group treated with *Lallemantia royleana* seed extract and Rifadin. After 30 days, the animals were sacrificed, the kidneys were collected, and placed in a 10% formalin fixative. Slides were prepared for histological examination and to detect the expression of BAX protein by IHC technique.

Results. The results showed, there are many pathological and inflammatory changes in group treated with Rifadin (1.5mg/kg/day) compared with control group, these changes represented by severe congestion of blood vessels and hemorrhage in cortex and medulla regions of kidney with appearance of fibrosis and infiltration of leukocytes. In the group that treated with *Lallemantia royleana* seeds extract there was no disintegration observed in the kidney's tissue with moderate congestion and hemorrhage, while there was moderate disintegration in the kidney tissue with mild to moderate congestion, hemorrhage and leukocytes infiltration in albino mice that treated with Rifadin and *Lallemantia royleana* (Balangu) seeds extract together. The

expression of BAX protein ranged from strong, mild, moderate immune reaction between the groups respectively compared with the control group.

Conclusion. The conclusion of this research is that the alcoholic extract of *Lallemantia royleana* seeds plays an effective and protective role in safeguarding kidney tissue from the harmful effects of Rifadin.

Keywords: kidney; Rifadin; *Lallemantia royleana*; BAX protein; inflammatory response

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Introduction

Rifadin, also called Rifampicin, Rifampin, and Rifaldazine [1], is a bacterial antibiotic used in the treatment of tuberculosis (TB), which is caused by *Mycobacterium tuberculosis*. Rifadin has the molecular formula $C_{43}H_{58}N_4O_{12}$ and a molecular weight of 822.59, with the synthetic formula being 3-4 (4-methyl piperazinyl-imino methylene) [2]. Rifampicin inhibits microbial RNA polymerase and prevents the synthesis of ribonucleic acid in bacterial cells [3]. Several studies have indicated the relationship between Rifadin and the toxicity of various organs, such as the spleen, bones, blood, reproductive organs, liver, and kidneys [4, 5, 6]. Other studies have indicated that 66% of patients treated with anti-tuberculosis drugs suffer from acute kidney failure [7], and the combination of isoniazid and Rifadin has been shown to cause kidney dysfunction [8] and oxidative damage [9].

Studies have shown that plant extracts can protect against diseases and have a positive impact on health, such as inducing antioxidant enzymes [10]. *Lallemantia royleana* (Balangu) seeds possess numerous pharmacological properties, including antioxidant, antidepressant, antiemetic, hypolipidemic, antimicrobial, and sedative properties [11]. The seeds of *Lallemantia royleana* are utilized in alternative medicine as a treatment for stomach diseases [12], cough suppression, urinary tract troubles, abscesses, and infections. The alcoholic extract of the seeds of *Lallemantia royleana* has shown antibacterial properties against bacterial strains such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, and *Enterobacter cloacae*, according to Sardarodiyani et al. [13]. The seeds of *Lallemantia royleana* (Balangu) contain green oil, which consists of a group of fatty acids, including linolenic acid, oleic acid, and palmitic acid [14].

The aim of the study: The aim of this study is to examine the effects of *Lallemantia royleana* (Balangu) seeds extract in modifying the inflammatory response and BAX protein expression as a marker of cell death in albino mice treated with Rifadin.

Materials and methods

The extraction of Balangu seeds:

The *Lallemantia royleana* (Balangu) seeds were obtained from the local market and ground until a powder was obtained. A sexual apparatus and 95% methanol were used to prepare a 1% weight/volume (50/500) oily extract of Balangu seeds with a clear green color [15].

The experiments steps:

This study included 24 male Swiss albino mice, 5 weeks old, with a weight range of 27 g. The mice were divided into four groups, with 6 animals in each group:

1. Control group, which was orally administered with 0.1 ml of normal saline (0.09%) for 30 days.
2. Group treated with 0.1 ml of Rifadin drug (1.5 mg/kg/day) for 30 days.
3. Group treated with 0.1 ml of *Lallemantia royleana* seeds extract (1%) for 30 days.
4. Group treated with 0.1 ml of *Lallemantia royleana* seeds extract (1%) and 0.1 ml of Rifadin drug (1.5 mg/kg/day) for 30 days. [16].

The histological slides preparation:

After 30 days, the animals were sacrificed. The kidneys were collected and placed in fixative (formalin 10%). The histological preparations, which included dehydration, clearing, infiltration, embedding, mounting on glass slides, and staining with H&E, were based on Suvarna et al. [17]. In the Immunohistochemical (IHC) study, the positively charged slides were used instead of normal glass slides for the mounting process, and the slides were treated with a special kit to detect Bax protein (SANTA CRUZ, USA) based on the process of Taylor and Rudbeck [18]. The BAX expression was evaluated by scoring 1000 cells randomly for each group sample to calculate the percentage of BAX using the following equation:

BAX expression (%) = (number of cells with BAX positive) / (total cells, i.e., cells positive with BAX + cells negative).

The statistical analysis was performed using SPSS. A comparison was made between the treated groups and the control group, and between the groups themselves, using One-Way ANOVA. The results were considered statistically significant at $P < 0.05$.

Results and discussion

Control group

Histological sections of this group appeared with a normal histological structure, represented by a kidney surrounded by a capsule consisting of loose connective tissue. It is differentiated into an outer region called the cortex, which contains renal corpuscles and sections of proximal and distal convoluted tubules, and an inner region called the medulla, which showed sections of thick and thin segments of Henle's loop as well as sections of collecting ducts (Fig. 1).

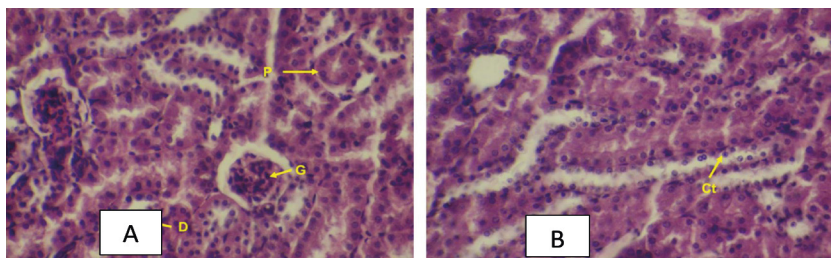


Fig. 1. A - Cross section of the kidney in the control group showed cortex components such as the proximal convoluted tubule. B - Cross section of the kidney in the control group showed the collecting tubule (Ct) in the medulla region, proximal convoluted tubule (P), distal convoluted tubule (D), glomeruli (G), and collecting tubule (Ct) H&E. (X 40).

Group administered with Rifadin 1.5mg/kg/day

The examination of kidney sections of male mice administered with 1.5 mg/kg/day of Rifadin for 30 days revealed many pathological and inflammatory changes compared with the control group (Fig. 1). These changes were represented by severe congestion of blood vessels and hemorrhage in the cortex region, extending into the medulla region. There were also very clear vacuoles in the renal tubules, especially in the area near the capsule, as well as clear disintegration in the tissue in both the cortex and medulla regions. Additionally, some renal glomeruli were lost, while others showed shrinkage or contraction. There was the appearance of fibrosis, infiltration of leukocytes between the renal tubules, and edema with the expansion of the lumen or cavities of the renal tubules (Fig. 2).

The severe congestion occurred as a result of tissue cell damage and death, which stimulates an inflammatory response due to the infiltration of white blood cells to remove and clean the tissue from the damaged cells [20]. This was observed in this study, where there was an infiltration of white blood cells in the areas between the urinary tubules in the kidney tissue in animals treated with

Rifadin (1.5 mg/kg/day). Previous studies have shown that drugs induce damage in kidney tissue at the glomerular, tubular, or interstitial levels [21]. These damages resulting from the effect of the drug may induce oxidative stress [22], as well as mitochondrial dysfunction due to the increased concentration of calcium ions, which leads to the removal of ATP, causing cell death [23]. The results of this study showed the presence of vacuoles in the tissue, especially in the cortex area adjacent to the renal capsule in animals treated with Rifadin. The presence of vacuoles resulted from the accumulation of fats in the cells and inhibition of fat metabolism and oxidation, which resulted from the generation of free radicals and a decrease in ATP levels due to the destruction of mitochondria [24]. The loss of glomeruli in the renal corpuscles and shrinkage of others resulted from the kidney's behavior to prevent the drug from reaching the bloodstream and limiting its harmful effects [25]. Miller et al. [26] indicated that daily or intermittent treatment with Rifadin is responsible for causing acute renal failure, which develops into chronic failure in patients treated with this drug.

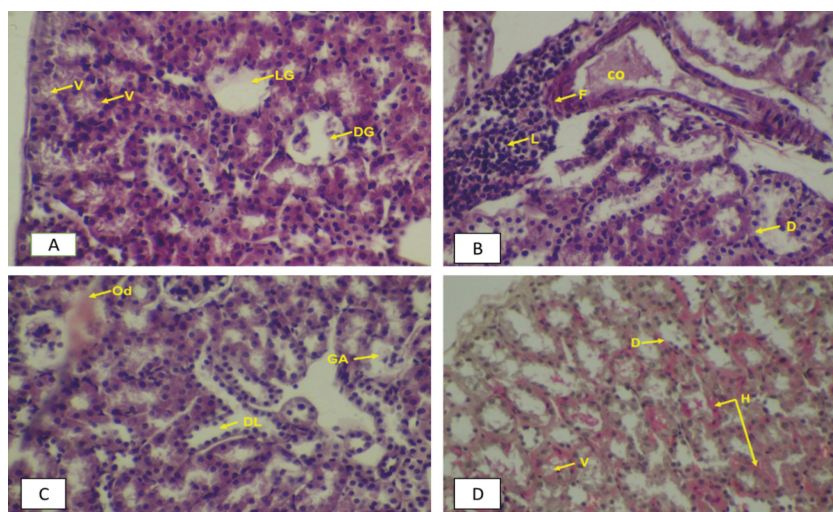


Fig. 2. Cross-section of the kidney in the group administered with 1.5 mg/kg/day Rifadin showed: A. Very clear vacuoles (V) in the renal tubules near the capsule, loss of glomeruli (LG), and damage (DG) to some renal glomeruli. B. Fibrosis and infiltration of leukocytes (L) between the renal tubules, as well as degeneration of the epithelium lining the renal tubules (D). C. Dilatation of the lumen in renal tubules (DL) and glomerular atrophy (GA), as well as edema (OD). D. Hemorrhage in the cortex region reaching the medulla region (H), vacuoles (V), and renal tubule degeneration (D). Stained with H&E (40X).

Group administrated with alcoholic extract (1%) of *Lallemantia royleana* (Balangu) seeds

The structure of the kidney tissue in this group appears similar to the normal structure of the control group in terms of the lack of disintegration of the kidney tissue and differentiation of the tissue into the cortex and medulla regions. Also, vacuoles, moderate congestion, and minimal hemorrhage between the renal tubules in the cortex and medulla regions were observed. No edema was observed with moderate infiltration, while no such changes were observed in the kidney tissue of the control group (Fig. 3).

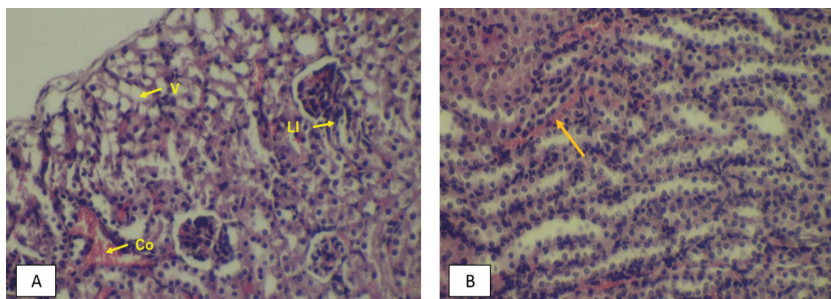


Fig. 3. Cross-section of the kidney in the group administrated with 1% alcoholic extract of *Lallemantia royleana* seeds showed: A - vacuoles (V), blood vessel congestion (Co), leukocyte infiltration (LI) in the cortex region; B- hemorrhage () between renal tubules in the medulla region. Stained with H&E. (40X).

The decrease in the histological changes in this group is due to the active components of *Lallemantia royleana* seeds extract, such as linoleic acid (Omega 3), in addition to its content of alkaloids, polyphenols, flavonoids, and tannins, which represent effective antioxidants that protect cells from the harmful effects of free radicals [27]. The presence of moderate congestion in the kidney tissue of this group and the subsequent infiltration of white blood cells may have resulted from the effect of the active components in the extract, which stimulate the release of inflammatory cytokines such as TNF- α and attract neutrophils and lymphocytes from peripheral blood [25].

Group administrated with alcoholic extract of *Lallemantia royleana* seeds (1%) and Rifadin (1.5 mg/kg/day)

The results of the microscopic examination of the kidney sections of this group showed moderate disintegration within the kidney tissue between the cortex and medulla regions, with congestion of blood vessels and slight hemorrhage in the cortex region. Additionally, there was an expansion of the lumen

of some proximal and distal convoluted tubules and the appearance of clear hemorrhage within the renal glomeruli, compared with the kidney tissue in the control group.

In contrast to the group of mice given just Rifadin, this group had moderate infiltration of leukocytes and a lack of damaged cells within the kidney tissue (Fig. 4).

The decrease in the pathological changes in the kidney tissue of this group may be due to the active components of the seed extract, like linoleic acid and phenols, which in turn have a role in raising the level of glutathione. This enhances drug metabolism and removes oxidative stress by converting free radicals into non-toxic substances, protecting the cells from the harmful effects of the drug [27].

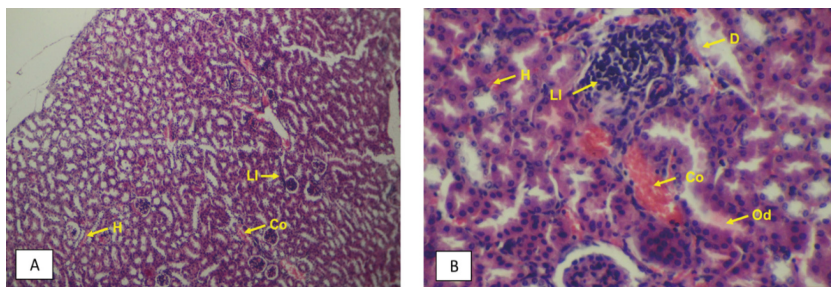


Fig. 4. Cross section of the kidney in the group administered with alcoholic extract of 1% Balangu seeds and 1.5 mg/kg/day Rifadin showed: A - Disintegration within the kidney tissue between the cortex and medulla regions with congestion (Co) of blood vessels and a little hemorrhage (H) between tubules in the cortex region, as well as leukocyte infiltration (LI). Stained with H&E (10x). B - Blood vessel congestion (Co), edema (Od), a little hemorrhage (H) between renal tubules, degeneration of renal tubules (D), and moderate infiltration of leukocytes (LI). Stained with H&E (40x).

The expression of BAX protein in the tissue of kidney

The results of microscopic examination of kidney tissue stained by IHC technique for detecting the expression of BAX protein, which predisposes the process of cell death, revealed a strong positive immune reaction in cells with BAX protein in the group of mice treated with Rifadin (1.5 mg/kg/day) compared with the other experimental groups. Meanwhile, the results showed a mild positive immune reaction to BAX protein in the group of mice treated with the alcoholic extract of *Lallemantia royleana* (Balangu) seeds (1%) and Rifadin (1.5 mg/kg/day) together compared to the Rifadin-only group. The results also

showed moderate or low positive immune reaction to BAX protein in the control group and the group of mice treated with the alcoholic extract of *Lallemantia royleana* (Balangu) seeds (1%) (Fig. 5).

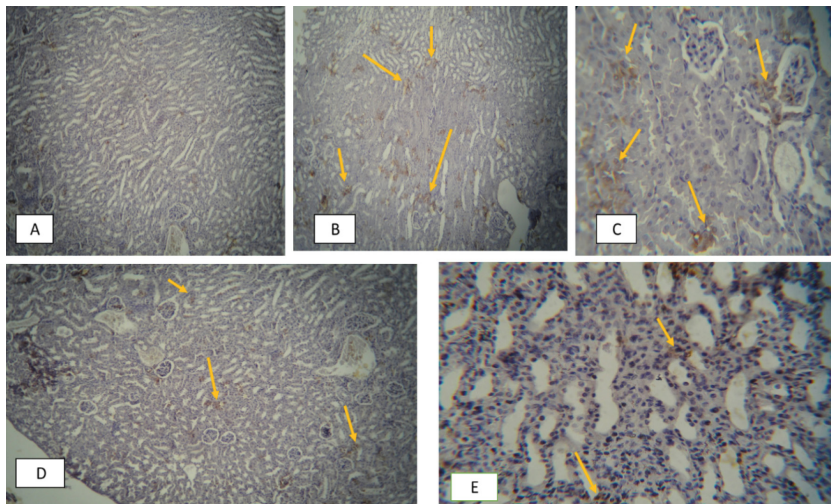


Fig. 5. Cross sections of the kidney in albino mice show the positive reaction of BAX protein: A - Control group (10x). B - Strong positive reaction of BAX in the group treated with Rifadin (10x). C - Strong positive reaction of BAX in the group treated with Rifadin (40x). D - Mild positive reaction in the group treated with Rifadin and the seeds extract of *Lallemantia royleana* (10x). E - Moderate positive reaction in the group treated with the seeds extract of *Lallemantia royleana* (40x). Area colored with DAB chromogen.

The high expression of BAX protein resulted from the effect of Rifadin, which enhances cell death, especially with multiple doses, by stimulating the release of cytochrome P450, formation of free radicals, destruction of mitochondria with the release of cytochrome C, and activation of the Caspase-3 pathway, which causes cell death [28].

The percentage of BAX protein expression showed a significant increase ($P < 0.05$) in the BAX expression percentage in the group of mice treated with Rifadin (1.5 mg/kg/day), which reached 13.200 ± 0.78 compared with other groups (control, the group treated with the seeds extract, the group treated with the seeds extract and the drug together), which had percentage values of (3.401 ± 0.11 , 5.344 ± 0.60 , 8.203 ± 0.30) % respectively. The results showed a non-significant increase in the percentage of expression between the control

group and the group treated with the seeds extract, while there was a significant increase ($P < 0.05$) between the group treated with the seeds extract and the group treated with the seeds extract and Rifadin together (Table-1). The decrease in the percentage of BAX expression in the kidney tissue of animals treated with both the drug and extract together, and extract alone, was consistent with the results of histological examination of the kidney in these groups. The extract of *Lallemantia royleana* seeds contains a mixture of active compounds that work synergistically to remove oxidative stress, neutralizing free radicals and protecting cells from entering the death pathway [29].

Table 1.

The percentage of BAX expression ($\% \pm SD$) in the kidney tissue of experimental groups

Experiment groups	BAX protein expression ($\% \pm SD$)
Control	3.401 \pm 0.11
Rifadin drug(1.5 mg/kg/day)	13.200 \pm 0.78**
(<i>Lallemantia royleana</i>) seeds extract (1%)	5.344 \pm 0.60
(<i>Lallemantia royleana</i>) seeds extract (1%) and Rifadin drug(1.5 mg/kg/day)	8.203 \pm 0.30*

*Significant at $p < 0.05$

Conclusion

Thus, the conclusion of this study is that the protective effect of the alcoholic extract of *Lallemantia royleana* (Balangu) seeds against the harmful effects of Rifadin is demonstrated by a decrease in inflammation signs, tissue damage, and the expression of BAX protein, which indicates a reduction in the number of cells undergoing death.

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Conflict of interest information. None declared.

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