Aqueous Extract of Boswellia against Rifampicin Toxicity in rats

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ABSTRACT
The aim of this study is to evaluate the rifampicin toxicity in albino rat’s liver and kidney and the reverse effect of boswellia plant extract. The study was conducted on twenty-four albino rats, maintained in animal house in college of pharmacy of Kerbala University in fourteen consequent days. The study was designed to evaluate hepatotoxicity in four albino rats’ groups: control, boswellia plant drenched, parenteral rifampicin and rifampicin and boswellia groups. Biochemical investigations were achieved to evaluate the liver toxicity, by measuring liver enzyme, blood total protein and albumin, urea and creatinine in addition to liver, kidney and lung histopathological studies. Biochemical studies show decrease in the hepatic enzymes and renal function tests parameters. This mean it has protective effects against rifampicin toxicity. At the same time, Histopathological observations found that less inflammation and deterioration in the tissues of boswellia treated animals compared with animals fed with rifampicin only. Boswellia can reduce and protect the animal from the renal and hepatic toxicity resulting from rifampicin long used.

INTRODUCTION
Rifampicin is a known antibiotic that is utilized in treating tuberculosis and it is extremely lowering duration of therapy and it is applied in the disposal meningococcal infections from carriers, as well in the treatment of leprosy [1][2]. Rifampicin conducts its effect by protein synthesis suppression in susceptible cells by prevention the initiation of transcription through binding to subunit of bacterial RNA polymerase. [3] Rifampicin is afinity to temporal rise in serum bilirubin and aminotransferase levels and it is a recognized purpose of clinically obvious acute liver disease that is could be serious or regrettably lethal. [4] combined designs of management with other drugs like isoniazid or pyrazinamide could rise this hazard. [5] Rifampicin prohibits DNA dependent RNA polymerase of microorganisms by the formation of constant enzyme – drug complex, procuring to prohibition of the inception of chain structuring in RNA structuring. [6][7] Rifampicin is efficiently absorbed orally and broadly distributed through almost each body fields and fluids and can cross placenta and may excreted in breast feeding milk and it is broadly excreted in bile and only little amounts is eliminated in urine without change. [8] On other hand, adverse effect of rifampicin includes nausea, vomiting, appetite loss stomach cramps and diarrhea. [9] other recognized side effects comprise dizziness, headache, fatigue and confusion, menstrual disturbance. [10] Immunoallergic representation and hepatic type that is dose dependent are the prevalent sorts of rifampicin toxicity. Interrupted and extended therapy are commonly related to the immunoallergic effects. Rifampicin results in cholestasis at sinusoids and moreover at liver canaliculi caused by defect in hepatocytes uptake and excretion. Hepatitis takes place in less than one percent of patients that are commonly have previously liver diseases. [11] Nephrotoxicity seems to be related to hypersensitivity reaction and commonly arise after intermittent or interrupted treatment that is proposed to be imputed to its metabolite desacetylriafampicin. [12] Hepatic injury can be assorted into cholestatic, hepatocellular and mixed perceived as rise in alanine aminotransferase more than two to three times and or rise in alkaline phosphatase more than two times its upper normal limit. [13][14][15] Drug metabolites created in liver by biotransformation can result in liver damage through the creation of toxic or reactive materials like electrophilic chemicals or free radicals and then destruct variable chemical reactions and hence cause necrosis or apoptosis. [16][17] Rifampicin produces hepatotoxicity through the action of oxidative strain that is has seen earlier in experimental rats. [18] rifampicin induces cytochrome P450 activity substantially and enhances the covalent binding of acetyl hydrazine reactive metabolites converting them to liver cells macromolecules that procure destruction of liver cells. [19][20]. Elongated exposition to rifampicin considerably diminishes glucose 6 phosphatase efficiency that is the impulse for lipid peroxidation increment. [21][22] phospholipids membrane degeneration enhanced by the activity of phospholipase A2 that is induced by the rise of intracellular calcium concentration caused by treatment with rifampicin [23][24] furthermore, fatty acid precipitation in liver and activation of CYP2E1 caused by too much lipid supply to the liver has been certified in drug induced liver injuries [25][26][27]. Boswellia carterii is a tree belongs to Burseraceae family had described in traditional Unani medicine versions as efficient treatment of an assortment of inflammatory diseases. [28][29] Dried resins of Boswellia gets an impact on inflammatory diseases by inhibiting the enzyme 5 lipoxygenase. [30] Boswellia performs particularly as leukotriene synthesis inhibition by straightforward on 5 lipoxygenase or translocation prevention. [31] furthermore, boswellia has properties to heal wounds and support respiration by its chemical ingredient like arabinose, terpenoids xylose, beta sitosterin and volatile oils. [30]
The resinous part comprises of rich terpenes among which are medicinally important group of boswellic acid (BAs) as pentacyclic triterpenic acid with four major pentacyclic triterpenic acid like beta-boswellic acid and its derivatives. Boswellic acid prohibits leukotrienes B\textsubscript{4} in rats that is a substantial inflammatory mediator in peritoneal neutrophil. [32] Moreover, Boswellia possess an evident analgesic effect on experiment rats by reducing spontaneous motor activity. Furthermore, it stabilizes mast cell in a dose dependent pattern and hence relieve anaphylaxis. [32] Boswellia extract has the ability to lowering the total cholesterol level and raise high density lipoprotein level in rats and similar results were attained in patients of type tow diabetes in addition to lowering SGOT and SCPT when it used for six weeks. [33] Boswellia acid minimizes liver malonaldehyde level and raises the nuclear factor erythroid 2 expression and hence, start hemeoxygenase 1 furthermore expression. It induces Geno defense effect by lowering cleaved caspase three protein expression and thence decreases DNA destruction, so, throughout these antioxidant properties, boswellia prevent hepatic apoptosis. [34] Also Boswellic acid augments the expression of nuclear factor erythroid 2 and hemeoxygenase1 and it expresses antioxidant action [34] Also it clears the reactive oxygen species and diminishes lipid peroxidation and DNA destruction of drug induced hepatotoxicity. [34] Gum extract stabilizes hepatocyte membrane and block leakage of SGOT, SGPT and alkaline phosphatase enzymes and decrease albumin levels and stop hepatic toxicity by blocking the free radicals and stabilization of endoplasmic reticulum. [35] Based on the importance of Boswellia gum, it was suggested for studying the reverse effect against rifampicin toxicity in albino rat's liver and kidney.

**MATERIALS AND METHODS**

**Extraction of Boswellia Gum**

Boswellia aqueous extract prepared by maceration of 100 g of grinded gum of boswellia carterii in 1000 ml distilled water for 24 hours then stored in refrigerator and used for oral drench.[36]

**Experimental Design**

The study was conducted on 24 albino rats. They were separated into four groups; each group consist of six animals maintained in animal house in college of pharmacy of Kerbala University with free access with free access to food and water administration. Groups are:

- Control group: intraperitoneal injected with 1 ml/Kg/day of DMSO for 14 days.
- Plant group: drenched 400 mg/Kg/day of boswellia extract for 14 days.
- Drug group: intraperitoneal injected with 120 mg/Kg/day of rifampicin for 14 days.
- Drug + plant group: intraperitoneal injected with 120 mg/Kg/day of rifampicin for 14 days.

Rifampicin was applied with a dose 120 mg/Kg/day, and the plant used was boswellia distilled water extract at dose 400 mg/Kg/day. Rifampicin was dissolved in DMSO solvent before injection for each animal using insulin syringe, while boswellia extract was administered orally by needle gavage. The animal was observed in their cages for clinical symptoms daily. At the end of experimental period, the animals were anesthetized by chloroform inhalation and blood was collected by cardiac puncture for serum biochemical analysis.

**Statistical analysis**

The results were represented as mean ± SE. differences between control and other experimental groups were tested for statistical significance using SPSS version 20 one-way analysis of variances (ANOVA), differences exist at \( P < 0.05 \).

**RESULTS**

**Biochemical changes among study groups**

A significant elevation in the values of ALP, AST, ALT, urea, creatinine, direct bilirubin, Indirect bilirubin &Total serum bilirubin was noticed in Rifampicin treated group, while the values of biochemical parameters of boswellia and rifampicin treated group are reduced significantly while the total protein level and albumin increased as shown in tables (1) and (2), and figures (1-10).

### Table 1. Show the effect of Boswellia carterii against Rifampicin toxicity on liver and kidney parameters of rats

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Creatinine</th>
<th>Urea</th>
<th>TSB</th>
<th>D</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>0.661±0.051a</td>
<td>29.333±2.996a</td>
<td>0.500±0.051a</td>
<td>0.133±0.021a</td>
<td>0.366±0.042a</td>
</tr>
<tr>
<td>Plant</td>
<td>0.741±0.053a</td>
<td>30.000±3.119a</td>
<td>0.400±0.044a</td>
<td>0.100±0.001a</td>
<td>0.300±0.044a</td>
</tr>
<tr>
<td>Drug</td>
<td>2.533±0.099b</td>
<td>68.200±2.463b</td>
<td>2.750±0.229b</td>
<td>1.266±0.187b</td>
<td>2.325±0.157b</td>
</tr>
<tr>
<td>Drug+plant</td>
<td>1.216±0.070c</td>
<td>4.1783±3.373c</td>
<td>1.200±0.103c</td>
<td>0.400±0.057a</td>
<td>1.166±0.177c</td>
</tr>
</tbody>
</table>

### Table 2. Show the effect of Boswellia carterii against Rifampicin toxicity on liver parameters of rats

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>ALT</th>
<th>AST</th>
<th>ALP</th>
<th>TP</th>
<th>Alb</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMSO</td>
<td>36.833±1.939a</td>
<td>44.166±1.641a</td>
<td>47.333±4.514a</td>
<td>4.663±0.275a</td>
<td>3.616±0.168a</td>
</tr>
<tr>
<td>Plant</td>
<td>37.166±2.257a</td>
<td>48.166±2.182a</td>
<td>46.500±5.175a</td>
<td>4.695±0.355a</td>
<td>3.133±0.260a</td>
</tr>
<tr>
<td>Drug</td>
<td>98.816±5.348b</td>
<td>164.000±3.405b</td>
<td>186.666±11.572b</td>
<td>3.066±0.252b</td>
<td>2.108±0.134b</td>
</tr>
<tr>
<td>Drug +plant</td>
<td>64.000±2.160c</td>
<td>102.083±4.332c</td>
<td>109.500±5.590c</td>
<td>4.204±0.361a</td>
<td>3.516±0.199c</td>
</tr>
</tbody>
</table>
Figure (1): Creatinine in control, plant, drug and drug + plant groups

Figure (2): Urea in control, plant, drug and drug + plant groups

Figure (3): Total serum bilirubin in control, plant, drug and drug + plant groups

Figure (4): Direct bilirubin in control, plant, drug and drug + plant groups

Figure (5): Indirect bilirubin in control, plant, drug and drug + plant groups

Figure (6): ALT in control, plant, drug and drug + plant groups
Figure (7): AST in control, plant, drug and drug + plant groups

Figure (8): ALP in control, plant, drug and drug + plant groups

Figure (9): Total protein in control, plant, drug and drug + plant groups

Figure (10): Albumin in control, plant, drug and drug + plant groups
Histopathological study of tissue slides:
Rifampicin group (Drug group)
In this group the liver section showed capsule thickening, fibro inflammation, mild interstitial inflammation and congestion, no fibrosis. Image (1)

While in the kidney section the tissue was normal and there was no remarkable pathology. Image (2).
The lung section in this group showed focal emphysematous changes with mild bronchiolitis and congestion. Image (3)

Rifampicin and Boswellia group (Drug + plant group)
In this group there was no remarkable pathology seen in the liver section. Image (4). The kidney section showed intact glomeruli and collecting duct and no remarkable pathology. Image (5).
The lung section also showed intact bronchiole and alveoli and no remarkable pathology. Image (6)
Image (4): Cross section of liver drug + plant group.

Image (5): Cross section of kidney drug + plant group.

Image (6): Cross section of lung drug + plant group.
DISCUSSION
The hepatotoxicity that induced by rifampicin scrutinized based on blood parameters after parenteral administration for fourteen days. Hepatic definite damage is indicated by the activity of alanine aminotransferase in rifampicin treated rat in comparison to the control group. Another parameter measuring hepatic damage is the activity levels of aspartate transaminase that significantly increased, this enzyme is more copious in the hepatocytes than in any different body cells, hence, it is utilized essentially as liver diseased scores. These enzymes are considered as a valuable indicator of livery injury since it is the main place for metabolism.[37]

Alkaline phosphatase activity is another indicator that increased in hepatic damage after rifampicin treatment with or without necrosis and seepage of intracellular contents into the blood stream.[38] Hyperbilirubinemia could be caused by rifampicin application where total serum bilirubin is increased that is caused by bilirubin uptake inhibition that gives rise to subclinical unconjugated hyperbilirubinemia. Conjugated hyperbilirubinemia is caused by bile salt exporter pump inhibition.[39]

Furthermore, serum bilirubin level is increased by inappropriate clearing bilirubin at the sinusoidal membrane or due to impeded release at the canalicular levels. [40][41] Albumin level is slightly diminished than normal and low albumin level can refer to liver disorder. [42] Also total protein was diminished in rifampicin treated liver tissues of rats as a consequence of decreased protein synthesis is hepatocytes. [43] Histologically, there are capsule thickening, fibro inflammation, mild interstitial inflammation and congestion in liver.

In our study there is significant elevation of creatinine and urea levels caused by kidney impairment when rats treated with rifampicin although, histological study reveals intact glomeruli and collecting tubes and no pathology is seen.

In the group that is treated with a drug and plant, there are significant reductions in enzymes level, urea and creatinine and with significant increase in the levels of protein and albumin in comparison with the group that is treated with rifampicin manifesting the protective effect of boswellia. The probable mechanism of boswellia protection effect is the stabilization of hepatocytes membrane and the prevention of leakage of intracellular contents including liver enzymes and alkaline phosphatase through prevent production of free radicals. [35]

CONCLUSION
in conclusion, the antitubercular drug rifampicin induces alterations in the level of protein, liver enzymes activity, bilirubin, urea and creatinine in the albino rat when parenterally administered in fourteen consecutive days and the boswellia leads to reverse these alterations.

REFERENCES


