Hepatoprotective activity of ethanol extract of *Ocimum basilicum* against CCl₄-induced hepatotoxicity in albino rats

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**ABSTRACT**

This experimental study was carried out to evaluate the hepatoprotective activity of the ethanolic extract of *Ocimum basilicum* whole plant that has been used in folk medicine in Sudan for the treatment of liver disorders. The extract was tested on rats at an oral dose of 200 mg/kg, 24h, after 36h samples were collected carefully; serum was separated by centrifugation (1200–1500 rpm for 15 min). EDTA was used as an anticoagulant in blood samples for hematological analysis, the tissues of liver were isolated carefully, fixed in 10% formalin and embedded in paraffin wax and they were taken for hepatoprotective effect on hepatocellular injury following post-treatment with carbon tetrachloride (CCl₄). Plasma levels of biochemical markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (Tbill) total protein (T.pro), albumin (ALB) and globulin were taken as biomarkers of liver damage. Also urea and creatinine were estimated. The ethanol extract at an oral dose of 200 mg/kg exhibited a significant (*P* < 0.001) protective effect by lowering serum levels of AST, ALT, and ALP comparable with that of silymarin used as a standard drug when related to CCl₄ (intoxicated) using spss. These biochemical observations were supplemented by histopathological examination of liver sections, which proved the protection effect of the plant extract. Preliminary phytochemical screening of the extract was conducted and the phytoconstituent identified include flavonoids, alkaloids, tannins, saponins, triterpens, sterols and cumarins.

**KEYWORDS:** *Ocimum basilicum* hepatoprotective activity, silymarin, carbon tetrachloride.

المستخلص

في هذا العمل تم تمت دراسة الأثر الوقائي للكبد لمستخلص الإبنائول لأحد النباتات السودانية التي تستعمل شعبياً لعلاج البرقان في السودان وهو نبات الريحان كاملاً في الجرذان البيضاء مخبرة (معطوبة) الكبد. أخذت عينات الدم والأنسجة وفصل المعمل بجهاز الطرد المركزي بعد 36 ساعة ثم تم اتباع الأثر الوقائي للمستخلص الإبنائول لنبات الريحان بجرعة 200 مجم / كجم24/ ساعة في الجرذان معطوبة الكبد بابة رابع كلوريد الكربون، حيث أن النبات احتفظاً كبيراً ومحفوظاً في انزيمات الكبد كما أحدث نوعاً ما في
INTRODUCTION

Traditional remedies have been using by many people around the world for the treatment of liver ailments for a long period of time without significant toxic effects. Therefore, it is necessary to search for complementary and alternative medicine, especially herbal drugs for the treatment of liver disease for better efficacy and safety to replace currently used drugs (1).

Plant *O. basilicum* belonging to the Lamiaceae family, commonly known as basil. It have been widely used in traditional Iranian medicine as a culinary herb and as a well-known source of flavoring principles (2). The antimicrobial and antioxidant activities of essential oils were obtained and have formed the basis of many applications, including fresh and processed food preservation, pharmaceuticals, alternative medicine and natural Therapies (3).

The activity of crude aqueous and ethanolic extracts of *Ocimum basilicum* were investigated against DNA viruses (herpes viruses (HSV), adenoviruses (ADV) and hepatitis B virus) and RNA viruses and results show and selected purified components, namely apigenin, linalool and ursolic acid, exhibit a broad spectrum of antiviral activities of extracts (1). sweet basil is mostly used to flavor and spice food and the leaf infusion has also been reported as being very effective against mild upper respiratory infections, broncho plasm and stress related skin disorder. The leaves in the form of paste are applied on cutaneous lesions and ring worm (4). In Africa, for example, it is used for treating whooping cough and various types of fever. The leaves are pulped in water to make ear- and eye-drops in parts of West Africa, and a leaf decoction is used for treating cough (5).

Furthermore both *O. basilicum* and its oil extract have received considerable attention for their potential therapeutic properties. These include hypoglucemic, hypolipidimic (6).

In Sudan the experimental study of essential oil of *Ocimum basilicum* suggested that basil is promising as repellents at 0.1% concentration against *Anopheles* mosquito and could be useful in the search for new natural repellent compounds (7).

The crude essential oil of wild Sudanese basil, assayed by the disc diffusion method, had three TLC-separated compounds which were active against *Salmonella typhimurium*. One of these was identified as geraniol, a major constituent of the essential oil. Thus basil essential oil has potential clinical or food applications as an antibacterial agent (8).

Objective of the present study was to investigate the hepatoprotective effect of the *O. basilicum* whole plant ethanolic extract against CCl4 liver damage in rats.

MATERIALS and METHODS

Plant material

The whole plant of *O. basilicum* was collected from local area Khartoum,
and shade dried at room temperature. The plant was identified and authenticated by the botanists in Medicinal and Aromatic Plants Research Institute (MAPRI). National Center of Research (NCR), Sudan.

The plant powder was extracted by maceration in ethanol 80%, the plant extract was evaporated to dryness at 40ºC by a rotary vacuum evaporator and the yield was calculated. The residue obtained was kept in dry clean bottles till used for pharmacological study(9).

**Phytochemical screening**

General screening was carried out to the method of Harbone(10) to determine the chemical constituents of each plant material.

Ten gram of the powdered part of each extract plants was refluxed with 100 ml of 80% ethanol for four hours. The cool solution was filtered to 100 ml. The prepared extracts (PE) were screened for the phytoconstituent.

**Experimental Animals**

Healthy adult albino rats of either sex weighing 120 – 130 g were obtained from the animal House at Faculty of Veterinary Medicine, University of Khartoum, Sudan. They were housed in specific standard laboratory conditions in (MAPRI) Khartoum, Sudan, and were kept in temperature controlled environment. All animals were fed with standard rat chow diet, medicinal with free access to water and received human care. The animals were given one week adaptation period before experimentation.

**Hepatoprotective activity**

24 adult Wistar albino rats were divided into four groups, with six animals in each group. Group I; the normal control group received three doses of 5% gum acacia mucilage (1ml/Kg, per oral) at 12 hour intervals (0 hours, 12 hour and 24 hours). Group II ;the rats of Carbon tetrachloride received three doses of vehicle at 12 hour intervals and injected subcutaneous a single dose of Carbon tetrachloride (1.25ml/kg) diluted in liquid paraffin (1:1) 30 minutes after the administration of the first dose of vehicle. While Group III; The test extract group administrated orally three dose of *o. basilicum* extract at a dose of 200mg /kg at 0 hour, 12 hour and 24 hour. Carbon tetrachloride was injected subcutaneous (1.25ml/kg) 30 minutes after the administration of the first dose of extract. group (IV) as a hepatoprotective drug control; rats were given three doses of Silymarin at a dose of (100mg/kg) at 0 hour, 12 hour and 24 hour. Carbon tetrachloride was injected subcutaneousa single dose (1.25ml/kg) 30 minutes after the administration of the first dose of silymarin.

After 36h all animals were sacrificed and samples were collected for test serobiochemical, hematological &histopathological investigations (11). Two groups of the study were compared, standard drug silymarin and the extract tested.

**Biochemical estimation**

The biochemical parameters were estimated using standard commercial kits. The parameters include the determination of Aspartate transaminase (AST), Alanine transaminase (ALT) according to the method of Reitman and Franke,(12); Alkaline phosphatase ( ALP) following the method of King (13), total protein measured by Lowery (14), albumin as described by Doumas (15) and bilirubin by the method of Malloy and Evelyn (16). Serum urea and Creatinine concentration was measured by an enzymatic method using a commercial kit. Globulin concentration was obtained by subtracting albumin concentration from that of total protein.
Hematological parameters
Hemoglobin concentration (Hb), packed cell volume (PCV), red blood cells count (RBC) and mean corpuscular haemoglobin concentration (MCHC), were measured using autoanalyzer.

Histopathological studies
After 36h of treatments the rats were sacrificed and the liver was isolated and immediately was fixed in 10% formalin and then embedded in paraffin wax. Sections of 4-5 microns thickness were made using rotary microtome and stained with haematoxylin-eosin and histological observations were made under light microscope(17)

Statistical analysis
The data were expressed as mean ± standard error of the mean (S.E.M). The Significance of differences among the group was assessed using SPSS followed by T-test.

RESULTS
Clinical findings
After injection of CCl₄, all treated groups suffered slight convulsion, depression. At necropsy, in group II (CCl₄ group) the liver showed fatty changes and slight increase in liver weights compared to the control groups. These changes were less noticed or disappeared in the extract treated groups and silymarin group.

Phytochemical screening
Preliminary phytochemical screening of whole plant of Ocimum basilicum, was revealed that the plant was richable in phytoconstituens of flavonoids. Tannins, Sterols, saponins, cumarins and triterpens.

Effect of Ocimum basilicum on liver function enzymes
There were significant increase (P<0.001) in the activity of the level of AST and Crea (P<0.05) in O.basilicum treated group, when compared to Silymarin and the other values of extract were found to be near to the Silymarin values. This indicates the protection effect of the plant. As shown in table (1) and figure (1).

Haematological finding
There were no differences between heamatological parameters of O.basilicum and Silymarin except in MCV value which was significantly decreased compared to Silymarin group. This result summarise in table (2).

Histopathological changes
In figure 2, in normal control rats (Group 1). Showed normal Hepatic architecture, rats received CCl₄ intoxicated rats (Group 2). Notice the lay centerilobule vacuoles and necrotic hepatocytes. Liver from rats received CCl₄ and O. basilicum (Group3) observed a sign of protection as it was evident by the less vacuolated hepatocytes and cellular regeneration. While rats given Silymarin and CCl₄ showed almost similar sign of protection (Group 4).

| Table 1: Effect of ethanolic extract Ocimum basilicum whole plant against CCL₄ – induced liver damage in rats on serum biochemical values (mean ± S.E): |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Silymarin plus carbon tetrachloride | AST (i.u/l) | ALT (i.u/l) | ALP (i.u/l) | Albumin (g/dl) | T.bill (g/dl) | D.bill (g/dl) | T.Prot(g/dl) | Globulin (g/dl) | Urea(m g/dl) | Crea(mg/dl) |
| 12±2 | 7.3±11 | 138±8 | 3.7±0.06 | 0.1±0.02 | 0.03±0.01 | 7±.06 | 3±0.2 | 42±4 | 0.3±.03 |
| Ocimum plus carbon tetrachloride | 18±5 | 7.1±9 | 140±9 | 3.8±0.3 | 0.1±0.02 | 0.02±.08 | 7±.06 | 3.2±0.4 | 46±3 | 0.48±0.03 |

Statistical analysis T- test (P<0.5), (P<0.01), (P<0.001) as compared to slymarin witho.basilicum
Table 2: Effect of ethanolic extract of Ocimum basilicum whole plant against CCL₄ – induced liver damage in rats on hematological values (mean ± S.E):

<table>
<thead>
<tr>
<th></th>
<th>PCV( %)</th>
<th>HB(g/dl)</th>
<th>RB C (10⁶ /mm)³</th>
<th>MCHC(g/l)</th>
<th>MCH(pg)</th>
<th>MCV(f l)</th>
<th>WBC(µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silymarin° plus carbon tetrachloride</td>
<td>12±2</td>
<td>7.3±11</td>
<td>9±1</td>
<td>3.7±0.06</td>
<td>0.1±.02</td>
<td>0.03±0.01</td>
<td>7±0.6</td>
</tr>
<tr>
<td>Ocimum plus carbon tetrachloride</td>
<td>18±5***</td>
<td>7.1±9</td>
<td>10±1</td>
<td>3.8±0.3</td>
<td>0.1±0.2</td>
<td>0.02±0.08</td>
<td>7±0.6</td>
</tr>
</tbody>
</table>

Statistical analysis T-test ° (P<0.5), °° (P<0.01), °°° (P<0.001) as compared to Silymarin.

Figure 1: Effect of O. basilicum whole plant ethanolic extract against CCL₄ – induced liver damage in rats on serum biochemical values (mean ± S.E)

Liver section showed normal Hepatic architecture.
Liver section showed normal CCL₄Hepatic architecture. CenterilubularVaculationwith
Liver section of *o.* *basilicum* group showed less hepatocytes vacuolation with cellular regeneration. Liver section of silymarin group showed less vacuolation of Hepatocytes with disorganization.

Figure 2: Histopathology effect of administration O. basilicum the whole plant ethanolic extract against CCl₄ induced liver damage in rats

**DISCUSSIONS**

The presence of jaundice is a cardinal feature of liver disease, and its presence usually signifies disturbance involving the hepatobiliary system[18]. CCl₄ was rapidly taken up, for example, by the brain and liver These organs' CCl₄ content then diminished, as CCl₄ was metabolized and redistributed to adipose tissue small amounts of CCl₄ entering the liver over the 2-hr infusion period.[19]. The evaluation of the preventive action in liver damage induced by CCl₄ has been widely used for hepatoprotective drug screening. CCl₄ is a widely used as experimental hepatotoxicant which requires metabolic activation by the liver cytochrome P-450 enzymes to form highly reactive hepatotoxic metabolites[20]. Damage to the structural integrity of liver is reflected by increase in the liver hepato-specific enzymes in the serum such as AST, ALT and ALP, because they are cytoplasmic in location and are released into circulation after cellular damage[21-23]. The level of bilirubin can be also used to assess liver function[24].

The ethanolic extract of *O. basilicum* (whole plant), when investigated for its hepatoprotective effect against CCl₄-induced liver damage at a dose of 200 mg /kg showed significant decrease in the levels of serum enzyme ALT, AST, ALP and T.bil, compared to the CCl₄ group. The effect of the extract of *O. basilicum* on the hepatic enzymes (ALT, AST and ALP) was almost similar to that of silymarin a known hepatoprotective agent[25].

The protective effect of the extract probably related to the antioxidant property of its high content of flavonoids, tanins, sterols and triterpens[26,27]. In addition the heamatological values of *O.basilicum* ethanolic extract was nearly to that of silymarin. Also *o.basilicum* extract masked CCl₄ – induced enlargement of the liver which confirmed the hepatoprotective effect of the plant extract. This indicating the production of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by the extract.

**CONCLUSIONS**

The above findings lead to the conclusion that the ethanolic extract of *Ocimum basilicum*, was exhibited a potential hepatoprotective activity against carbon tetrachloride induced hepatotoxicity and validate the traditional use of these plant in
hepatocellular jaundice. Further studies must be conducted such as concurrent treatment of the plant extract in providing the hepatoprotective activities to further elucidate the bioactive component of the plants, which could include flavonoids and Tanins and assess the mechanism of hepatoprotective action of the plant. Hence, one can suggest the inclusions of this plant in the management of liver disorders are justified.

REFERENCES:


