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Hepatoprotective Activity of Ethanol Extract of *Ocimum basilicum* against CCl_4– 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. Preliminary phytochemical screening of the extract was conducted to determine the active constituents. The extract was tested on rats at an oral dose of 200 mg/kg for hepatoprotective effect before injection of CCl_4 (0h) and following post- treatment with CCl_4 at 12 and 24h. CCl_4 was injected at 12 hour as single dose (1.25ml/kg). After 36h samples were collected for serobichemical and haematological investigations. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (Tbil), direct bilirubin (Dbil), total protein (TP), albumin (Alb) and globulin (Glo) were taken as biomarkers of liver damage. The tissues of liver were also isolated carefully for histopathology. Phytoconstituents identified in *O. basilicum* whole plant ethanolic extract included flavonoids, alkaloids, tannins, saponins, triterpens, sterols and cumarins. The ethanol extract at a dose of 200 mg/kg exhibited a significant ($P < 0.05$) protective effect by lowering serum levels of AST, ALT, and ALP comparable with that of silymarin used as a standard drug when related to CCl_4. Haematological parameters were also found similar to silymarin group. These biochemical observations were supplemented by histopathological examination of liver which proved to be protected by the plant extract.
The study concluded that *O. basilicum* ethanolic extract has a potential hepatoprotective activity against carbon tetrachloride induced hepatotoxicity in rats. This may be useful in treating liver disorders in man.
KEYWORDS: *Ocimum basilicum* hepatoprotective, silymarin, carbon tetrachloride.

المستخلص

هذه الدراسة التجريبية صممت لتقييم الأثر الواقى للكبد للمستخلص الإيثانولى لنبات الريحان كاملا والذى يستعمل فى الطب الشعبى فى السودان لعلاج مشاكل الكبد. تم عمل مسح كيميائى أولى للنبات لمعرفة المركبات الفعالة. تم اختبار المستخلص فى الجرزان بجرعة فمويه 200 ملجم/كجم لمعرفة الأثر الواقى للكبد قبل حقن رابع كلوريد الكربون (ساعة الصفر) وبعد حقن رابع كلوريد الكربون بعد 12 و24 ساعة. تم حقن رابع كلوريد الكربون بعد 12 ساعة جرعة واحدة (1.25 ملجم/كجم). بعد 36 ساعة تم تجميع العينات للفحوصات الكيميائية والدم. ALT AST, ALP, و البيلروبن الكلى والمباشر، البروتين الكلى، الالبيومين والقلوبيولين أخذت كعلامات كيميائية

لتخريب الكبد. تم عزل نسيج الكبد ايضا بحرص للفحص النسيجى. المركبات الكيميونباتية التى تم التعرف عليها فى المستخلص الايثيلى لنبات الريحان الكامل احتوت على الفلافونويدات , الالكالويدات ، التانينات، الصابونينات، التربينات الثلاثية، الاسترول و الكيومارينات . المستخلص الايثيلى بجرعة 200 ملجم/كجم اوضح تأثيرا واقيا معنويا بواسطة خفض مستويات مصل AST, ALT, ALP والبليروبين الكلى والمباشر مقارنة بعقار السليمارين المستعمل كعقار قياسى وعند مقارنتها بمجموعة رابع كلوريد الكربون . ايضا وجد ان فحوصات الدم شبيهة بمجموعة عقار السليمارين. هذه الفحوصات الكيميائية تم ربطها بالاختبارات النسيجية للكبد والتى اثبتت التأثير الواقى لمستخلص النبات. خلصت الدراسة الى ان مستخلص الريحان له نشاط واقى كبدي محتمل ضد رابع كلوريد الكربون المحدث لتسمم الكبد في الجرذان .قد يكون هذا مفيدًا في علاج اضطرابات الكبد لدى الإنسان.

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 *Ocimum basilicum* belonging to the *Lamiaceae* family, commonly known as rihan in Sudan, in English is known as basil [(2)]. It has been widely used in traditional medicine as a culinary herb and as a well-known source of flavoring principles [(3)].

The crude aqueous and ethanolic extracts of *O.basilicum* and its purified components, namely apigenin, linalool and ursolic acid, exhibited a broad spectrum of antiviral activities [(4)]. The essential oils of *O.basilicum* were found to have antimicrobial and antioxidant activities [(5)]. The leaf infusion has also been reported as being very effective against mild upper respiratory infections, bronchospasm and stress related skin disorder. The leaves in the form of paste are applied on cutaneous lesions and ring worm [(6)] .

In Africa, 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 [(7)] .

Furthermore, both *O. basilicum* and its oil extract have hypoglycemic and hypolipidemic effects [(8)] .In Sudan essential oil of *O. basilicum* suggested that basil is promising as repellents at 0.1% concentration against *Anopheles* mosquito and as an antibacterial agent [(9,10).]

The objective of the present study was to investigate the hepatoprotective effect of the *O. basilicum* whole plant ethanolic extract against CCl_4 induced liver damage in rats.

MATERIALS and METHODS

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

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 [(11)]. The residue obtained was kept in dry clean bottles till used for pharmacological study.

Phytochemical screening: General screening was carried out according to the method of Harbone [(11)] to determine the chemical constituents of the plant material. 10 g of the powdered part of the plant was refluxed with100 ml of 80% ethanol for four hours. The cool

solution was filtered and screened for the phytoconstituents.

Experimental Animals: Twenty four 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 with free access to water and received human care. The animals were given one week adaptation period before experimentation.

Hepatoprotective activity: A twenty four rats were divided into four groups, with six animals each. Group I; is left as control and received three doses of 5% gum a*cacia* mucilage vehicle at a dose of1ml/Kg orally at 12 hour intervals (0 , 12 and 24 hours). Rats in group II were injected with three doses of vehicle at 12 hour intervals and injected a single dose of carbon tetrachloride subcutaneously (1.25ml/kg diluted in liquid paraffin 1:1) 30 minutes after the administration of the first dose of vehicle. While rats in group III, were given orally three dose of *O. basilicum* extract at a dose of 200mg /kg at 0 , 12 and 24 hours. carbontetrachloride was injected subcutaneously (1.25ml/kg) 30 minutes after the administration of the first dose of the extract. Rats in group (IV), a hepatoprotective drug control, were given three doses of silymarin at a dose of 100mg/kg at 0, 12 and 24 hours. Carbon tetrachloride was injected subcutaneously at a single dose (1.25ml/kg) 30 minutes after the administration of the first dose of silymarin. Animals were sacrificed after 36h and samples were collected for serobiochemical, haematological and histopathological investigations.

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 was measured by Lowery method [(14),] albumin was analyzed as described by Doumas [(15)] and bilirubin by the method of Malloy and Evelyn [(16)] . Globulin concentration was obtained by subtracting albumin concentration from that of total protein.

Haematological parameters: Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were measured using automatic analyser (Humacount plus- Human GmbH Max- Planck-Ring21, D-65205 Wlesbaden, Germny).

Histopathological studies: After rats were sacrificed the livers were isolated and immediately fixed in 10% neutral formalin and then embedded in paraffin wax [(17)].

Statistical analysis: Data were analyzed using SPSS followed by T-test. The data were expressed as mean ± standard error (S.E).

RESULTS

Phytochemical screening: Preliminary phytochemical screening of the whole plant of *O. basilicum* revealed that the plant was rich in phytoconstituents such as flavonoids, tannins, sterols, saponins, cumarins and triterpens.

Clinical findings and necropsy: After injection of CCl_4, all treated groups suffered slight convulsion and depression. At necropsy, in group II (CCl_4 group) the livers were pale and enlarged. These changes were less

noticed in the extract treated group and silymarin group.

Effect of O. basilicum on biochemical parameters: In CCl_4 group, there were significant increase ($P<0.05$) in the activities of AST, ALT, ALP, Tbill and Dbill in compared control and treated groups. In *O. basilicum* and silymarin groups **t**here were significant decrease in the activities of AST, ALT, ALP, Tbill and Dbill compared to CCl_4 ($P<0.05$). There were significant increase ($P<0.05$) in AST level in *O. basilicum* treated group, when compared to silymarin and the other values of extract were found to be near to the values of silymarin group (Table1).

Table (1): Mean biochemical effect of ethanolic extract of *Ocimum basilicum whole plant against CCl_4 – induced liver damage in rats.*

Biochemical parameters	CCl_4	Control	*Ocimum*	Silymarin
AST (i.u/l)	46±32	15±6*	18±5*	12±2*
ALT (i.u/l)	34±2	3.5±4*	7.1±9*	7.3±11*
ALP (i.u/l)	260±8	111±3*	140±9*	138±8*
TP (g/dl)	8±0.4	6.5±0.5	7±0.6	6.7±0.2
Alb (g/dl)	4.1±0.1	3.6±0.2	3.8±0.3	3.7±0.06
Glo (g/dl)	3.7±0.4	3.4±0.6	3.2±0.4	3±0.2
Tbil (g/dl)	0.6±0.2	0.1±0.04*	0.1±.02*	0.1±.02*
Dbil (g/dl)	0.3±0.2	0.03±0.04*	0.02±.08*	0.03±0.01*

Statistical analysis T- test * ($P<0.05$) as compared to CCl_4 group. Values are expressed as mean±SE.

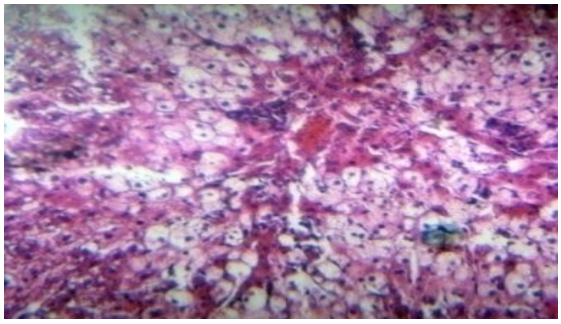
Haematological finding: There were no differences between haematological parameters of *O. basilicum* and silymarin except in MCV value which was significantly decreased compared to silymarin group. The result is summarized in Table (2).

Table (2): Mean haematological effects of ethanolic extract of O. basilicum whole plant against CCl_4 – induced liver damage in rats

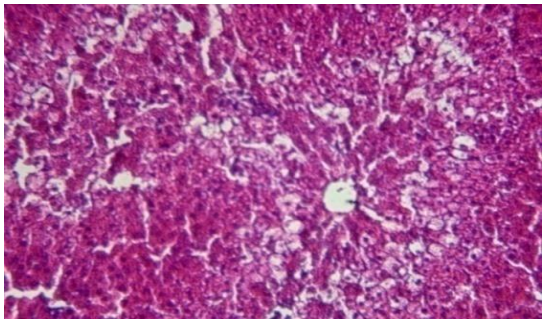
Haematological parameters	CCl_4	Control	*Ocimum*	Silymarin
WBC ($\times 10^3$ µl)	14±3	11±1	16±1	14±1
RBC (10^6 cells/µl)	10±0.4	9±0.2	10±1	9±1
HB (g/dl)	14±1	15±0.2	16±0.3*	15±1
PCV **(%)**	45±2	55±1*	55±2*	56±4*
MCV (f l)	55±1	60±1*	56±1	60±1*
MCH (pg)	16±0.4	12±1*	16±1	15±1
MCHC (g/l)	27.8±3	27.0±2	26.6±4	25.6±1

Statistical analysis T- test* ($P<0.05$) as compared to CCl_4 group. Values are expressed as mean±SE.

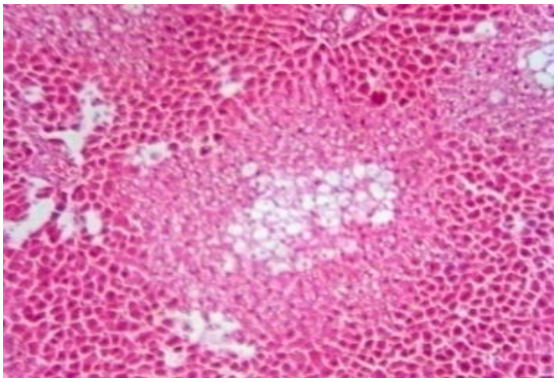
Histopatological changes: Liver sections of control rats (Group 1), showed normal hepatic architecture. Livers from rats received CCl_4 (Group 2), showed diffused centerilobular vacuoles and necrotic hepatocytes. In the liver from rats received *O. basilicum* ethanolic extract and CCl_4 (Group 3) there were less vacuolation in hepatocytes and cellular regeneration. Livers from rats given silymarin and CCl_4 (Group 4) showed less area of vacuolation and normal hepatocytes (Figure 1) .



Liver form rat received CCl_4 noticed diffuse centerilubular vacuolation with hepatic necrosis. H & E ×40.



Liver from rat treated with *O. basilicum* showed hepatocellular vacuolation with cellular regeneration. H & E ×40.



Liver from rat treated with silymarin showed less vacuolated areas of hepatocytes. H & E ×40.

Figure1: Histopathology of administration of O. basilicum the whole plant ethanolic extract against CCl_4 induced liver damage in rats

DISCUSSION

The present study was carried out to investigate the hepatoprotective effect of *O. basilicum* whole plant against CCl_4 induced liver damage in rats. This plant is used widely to treat various ailments including jaundice. Jaundice is a serious feature of liver disease, and its usually signifies disturbance involving the hepatobiliary system [18].

The evaluation of the preventive action in liver damage induced by CCl_4 has been widely used for screening of new hepatoprotective drug. CCl_4 is a widely used as experimental hepatotoxicant which requires metabolic activation by the liver cytochrome P-450 enzymes to form highly reactive hepatotoxic metabolites [1,18,19,20]. 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. The level of bilirubin can be also used to assess liver function [18,19,20, 21,22].

In this study, the ethanolic extract of *O. basilicum* (whole plant), when investigated for its hepatoprotective effect against CCl_4 induced liver damage at a dose of 200 mg/kg showed significant decrease in the levels of serum enzyme ALT, AST , ALP, Total and direct bilirubin compared to the CCl_4 group**.** The effect of the extract of *O. basilicum* on the hepatic enzymes (ALT and ALP) was almost similar to that of silymarin a known hepatoprotective drug. Similar findings were indicated by [23,24,25].

The protective effect of the extract probably related to the antioxidant property due to its high content of flavonoids, saponin, tannins, sterols and triterpens [23,26,27] and due to its superoxide radical and nitric oxide radical scavenging activities [23,27].

In addition the haematological values of *O. basilicum* ethanolic extract were nearly to that of silymarin. Also *O. basilicum* extract masked CCl_4 – induced injury and enlargement of the liver which confirmed the hepatoprotective effect of the plant extract. This indicating the production of structural integrity of hepatocyte cell membrane or regeneration of damaged liver cells by the extract.

CONCLUSION

The above findings lead to the conclusion that the ethanolic extract of *O. basilicum,* exhibited a potential hepatoprotective activity against carbon tetrachloride induced hepatotoxicity. 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 tannins 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.

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