Hepatoprotective effect of Basil Leaves (*Ocimum basilicum* L.) on CCl_4 induced liver toxicity in rats

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Abstract

The present study was designed to investigate the hepatoprotective and antioxidant effects of Basil Leaves (BL) on carbon tetrachloride (CCl_4) induced rats' hepatotoxicity. Animals were (28) albino rats divided into 4 groups. Group 1 was used as a control and received basal diet daily for the period. Group 2 (CCl_4 group) rats injected with CCl_4: corn oil (1:1) 1 ml/kg, s. c. on 15th day, 18th and 21st day. Group 3(BL protected group) rats feed diet supplemented with (1% (BL) /21days) then injected with CCl_4 dose as group 2. Group 4 (BL protected group) rats administered BL (2% BL/ 21 days) then injected with CCl_4: as group 2. Biological evaluation parameters were determined (IBW, FBW, FER, BWG%) for rats. Blood samples were separated into serum for liver functions determination (ALT, AST and ALP) and sample for antioxidant parameters determination (MDA and GPx), and lipid profile (TC, TG, HDL-c, LDL-c and VLDL-c). The increase in IBW, FBW, BWG% and FER were significantly ameliorated by the administration of Basil leaves treatments, compared to hepatotoxicity rats. The results revealed that in CCl_4 group, there were significant increases in ALT, AST, ALP, TC, TG, LDL-c, VLDL-c and MDA, while the parameters HDL-c and GPx showed significant decreases. Meanwhile, the administration of BL resulted in a significant decrease in all elevated parameters mentioned and an increase in HDL-c and GPx. Therefore, it could be concluded that BL had hepatoprotective and antioxidant role.

Key words: Basil, Leaves, CCl_4, liver, antioxidant, toxicity, hepatoprotective.

Introduction

Liver plays important metabolic, detoxification, and secretory roles in the body. Liver disease is associated with distortion of these metabolic functions **(Chaware et al., 2009)**. Humans are continuously exposed to different kinds of chemicals such as food additives, industrial chemicals, pesticides and other undesirable contaminants **(Gabele et al., 2003)**. Carbon tetrachloride (CCl4) is a common hepatotoxin that is widely used to induce toxic liver injuries **(Pereira-Filho et al., 2008)**. Search for newer drugs continues because the existing synthetic drugs have several limitations **(Gandhimathi and Kumar, 2012)**. Ocimum basilicum Extract **(OBE.),** sweet basil, or Rehan in Egypt is an annual, widely cultivated herb known for its medicinal value. Most biological activities like antioxidant effects are associated with its volatile oil content **(Shirazi et al., 2014)**. Th phytochemical analysis of the plant reveals that the plant is rich source of polyphenols **(Arts and Hollman, 2005)** which include flavonoids, phenolic acids, phenolic alcohols, stilbenes, and lignans **(D'Archivio et al., 2007).** In leaves extract, the total phenolic content has been found to be 32.23 ± 4.45 **(Rafat et al., 2010).** The aim of this study was to evaluate the hepatoprotective effect of ocimum basilicum in CCl4 induced hepatotoxicity as a standard drug through the investigation of liver functions (ALT, AST and ALP), antioxidant parameters (MDA, and GPx) and lipid profile (TC, TG, HDL-c, LDL-c and VLDL-c).

Materials And Methods

Materials

1. **Leaves of Basil** would be obtained from Pharmacy Farm, Cairo University, Egypt.
2. **Carbon tetrachloride CCl_4 and chemical kits** would be obtained from El-Gomhoriya Pharm, Cairo, Egypt.
3. **Casein, cellulose, sucrose, choline chloride, D-L methionine, vitamins and minerals** constituents would be purchased from El-Gomhoriya Pharm.Cairo, Egypt.

Methods

Preparation of Basal Diet:

The basal diet would be included protein (14%), fat (5%), mineral mixture (3.5%), vitamin mixture (1%), cellulose (5%), sucrose (10%), choline chloride (0.2%) and the remainder will be Corn starch. These constituents will be thoroughly mixed and formulated according to **(Reeves et al., 1993).**

Induction of hepatotoxicity in rats:

Carbon tetrachloride (CCl_4)-induced acute hepatotoxicity in rats. Intraperitoneal injection of male albino rats with CCl4 1mL/kg, (1:1) mixture with corn oil for 3 days increased serum alanine transaminase, aspartate transaminase, and alkaline phosphatase activities as well as total bilirubin, triglycerides and total cholesterol levels. This is in addition to the disrupted histology **(karthikeyan and Deepa, 2010).**

Experimental design:

Adult male albino rats Sprague Dawley Strain (28 rats) will be housed in well aerated cages under hygienic condition in lab of Agricultural Research Center-Giza. They will be left for one week as adaptation period and they will be allowed to feed standard laboratory food and water. After this week the rats would be divided into two main groups, as follows:

The first main group: Negative control group, rats (n=7) will be fed on basal diet.

The second main group: Hepatotoxic group, rats (n=21), would be injected 1 ml CCl_4/kg b.w. **(karthikeyan and Deepa, 2010)**. After 24h from injection for 3 days, rat from each group would be taken to measure liver function to be sure that all rats had liver injury. After liver injury rats will be divided as follow: -
Subgroup (1) Hepatotoxic rats (positive control group), animals will be fed on basal diet only.

Subgroup (2) Hepatotoxic rats would be fed on basal diet supplemented with (1% Basil leaves powder per kg of basal diet).

Subgroup (3) Hepatotoxic rats would be fed on basal diet supplemented with (2% Basil leaves powder per kg of basal diet).

Biological Evaluation

Determination of FI, IBW, FBW and Percent of BWG: Feed intake will be recorded daily, and animals will be weighed at the beginning and twice a week throughout the experimental period. Body weight gain% and feed efficiency ratio will be calculated at the end of the experiment according to the method of **(Chapman et al., 1959)**.

Biochemical Assessments

Evaluation of Lipid Profile: Serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-c), and low density lipoprotein cholesterol (LDL-c) were determined using trading reagent kits (Biomed diagnosis, Egypt) as referred by **Zollner and kirsch (1962), Vassault *et al.,* (1986), Hostmark *et al.,* (1991), Friadwald *et al.,* (1972) and Young, (2001),** respectively. While, very low-density lipoprotein cholesterol (VLDL-C) was measured using the following formula: **VLDL-c (mg/ dL) = TG/5**

Evaluation of Liver Functions: The serum activity of Alanine transaminase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) enzymes and gammaglutamyl (GGT) was colorimeters quantified utilizing kits (Diamond Co, Hanover, Germany) in line with the instructions of **Young (1997)** for AST and ALT assay, **Sherwin (1984)** for ALP assay and **Dufour *et al.,* (2000)** for GGT assay. The biometrics were quantified using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) adjusted at 505 for ALT, AST and ALP, and 510 nm for GGT.

Serum levels of total protein (TP), albumin (Alb), total bilirubin (TBL) and direct (DBL) were quantified colorimetrically using a spectrophotometer (Hum star 200, automatic biochemistry analyzer, Germany) as mentioned by **Tietz (1994)**, **Young (2000)**, **Henry (1991)** and **Burtis and Ashwood (1999)**, respectively.

Evaluation of Malondialdehyde and Activities of Antioxidant Enzymes: The principal method for the determination of oxidative stress was depending on colorimetric by quantifying thiobarbituric acid (TBA) reactivity as

malondialdehyde (MDA) in a spectrophotometer adjusted at 534 nm according to the described method by **Ohkawa *et al*., (1979)**.

The activities of antioxidant enzymes glutathione peroxides (GPx) was determined as referred by the commercial testing kits (Cayman Practice ELISA Kits). The serum activity of GPx was checked according to the kit's instruction manual as mentioned by **Beutler *et al*., (1963)** and **Paglia and Valentine., (1967)** using spectrophotometrically at 405 nm and 340 nm.

Statistical Analysis: Data were assessed statistically according to the computerized SPSS package program (SPSS 22.00 software for Windows) by one-way analysis of variance (ANOVA). The gained data was stated as Mean ± SD, and the significant difference between means was estimated at $p<0.05$**.**

Results and Discussion

Results

Effect of Basil leaves on FI, FBW, BWG and BWG (%) in rats with hepatotoxicity: The current results in Table 1 revealed that untreated hepatotoxicity rats (positive) have a significant ($P<0.05$) decrease in FI compared to that of healthy rats (negative rats). In contrast, hepatotoxicity rats fed on basal diet supplemented with (1% and 2% Basil leaves) had significant increase changes in FI compared to that of the positive rats and had a significant decrease, compared to the negative group.

Regarding body weight, the tabulated results showed that hepatotoxicity rats fed on basal diet had a significant ($P<0.05$) decrease in IBW, FBW and BWG%, compared to that of the normal rats fed on basal diet. Incorporated, the hepatotoxicity rats fed on basal diet supplemented with (1% and 2% Basil leaves) caused significant increase ($P<0.05$) in IBW, FBW and BWG%, compared to the positive control rats. On other hand, the result revealed that hepatotoxicity rats fed on basal diet had a significant ($P<0.05$) increase in FER compared to that of the normal rats fed on basal diet while, the hepatotoxicity rats fed on basal diet supplemented with (1% and 2% Basil leaves) caused significant increase ($P<0.05$) in FER compared to the positive control rats. The increase in IBW, FBW, BWG% and FER were significantly ameliorated by the administration of Basil leaves treatments, compared to hepatotoxicity rats.

Table (1): Effect of Basil leaves on FI, FBW, FER and BWG (%) in rats with hepatotoxicity

Parameters / Groups	Parameter as Mean ± SD				
	FI (g)	**IBW (g)**	**FBW (g)**	**FER**	**BWG (%)**
Negative group	20.25	190.2±4.55^{a}	219.0±4.88^{a}	3.16±0.24^{a}	15.14±1.10^{a}
Positive group	15.50	195.0±2.85^{a}	196.5±2.95^{b}	0.21±0.03^{d}	0.76±0.14^{d}
1 % Basil leaves	19.00	205.5±4.27^{a}	220.75±4.49^{a}	1.78±0.09^{bc}	7.42±0.37^{bc}
2 % Basil leaves	20.75	201.7±3.72^{a}	223.75±3.92^{a}	2.37±0.11^{ab}	10.91±0.21^{b}

Means with different letters in each row are significantly differs at $p< 0.05$; **FI:** Food Intake; **IBW:** Initial body weight**; FBW:** Final body weight**; FER:** Feed efficiency ratio**; BWG%:** Change in body weight gain%.

Effect of Basil leaves on TC, TG, LDL-c, HDL-c and VLDL-c in rats with hepatotoxicity: In the case of the serum lipid profile, the parameters of serum TC, TG, LDL-c, HDL-c, and VLDL-c levels were used to check the effect of Basil leaves on hepatotoxicity rats. The results in Table 2 revealed that hepatotoxicity rats fed on basal diet had a significant ($P<0.05$) increase in the serum concentrations of TC, TG, LDL-c, HDL-c, and VLDL-c, and a decrease in HDL-c levels, compared to that of the normal rats fed on a normal basal diet. In contrast, the administration of Basil leaves caused a significant amendment in the serum levels of the above parameters, as compared to hepatotoxicity rats fed on basal diet alone (positive rats).

The rate of improvement in the serum levels of TC, TG, LDL-c, and VLDL-c was more evident with the administration of 2% Basil leaves, while serum HDL-c, levels were improved in treated groups with same level 2% Basil leaves.

Table (2): Effect of Basil leaves on TC, TG, LDL-c, HDL-c and VLDL-c in hepatotoxicity rats

Parameters / Groups	TC (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Negative group	184.45±1.54^{g}	96.50±1.19^{f}	60.50±1.70^{ab}	104.65±0.83^{d}	19.30±0.23^{f}
Positive group	241.77±1.31^{a}	153.50±1.44^{a}	37.75±1.10^{c}	173.32±1.22^{a}	30.70±0.28^{a}
1 % Basil leaves	210.67±2.75^{c}	143.74±1.05^{b}	61.25±1.31^{ab}	120.67±1.83^{c}	28.74±0.21^{b}
2 % Basil leaves	203.92±1.46^{de}	129.79±0.88^{c}	58.25±2.28^{b}	119.71±3.73^{c}	25.95±0.17^{c}

Means with different letters in each row are significantly differs at p< 0.05; **TG:** Triglycerides; **TC:** Total Cholesterol; **LDL- C**: Low Density Lipoprotein; **HDL- C**: High Density Lipoprotein; **VLDL-C:** Very Low-Density Lipoprotein Cholesterol**.**

Effect of Basil leaves on AST, ALT and ALP in hepatotoxicity rats: The tabulated results in Table (3) outlined that untreated rats with (CCL_4) had a significant increase in the serum activity of AST, ALT and ALP enzymes, compared to the normal rats. Whilst the treated rats by the oral administration of Basil leaves caused significant (P<0.05) reductions in the serum activity of AST, ALT and ALP enzymes, compared to hepatotoxicity rats. The significant improvement in the activities of liver enzymes (AST, ALT and ALP) were reported in hepatotoxicity rats treated with 2% Basil leaves.

Table (3): Effect of Basil leaves on AST, ALT and ALP hepatotoxicity rats.

Parameters / Groups	ALT (u/L)	AST (u/L)	ALP (u/L)
Negative group	18.25±1.85^{e}	21.50±1.32^{f}	81.14±0.53^{e}
Positive group	40.05±1.06^{a}	41.75±0.85^{a}	117.00±1.08^{a}
1 % Basil leaves	29.98±0.90^{b}	36.65±1.02^{b}	96.25±1.31^{bc}
2 % Basil leaves	22.50±0.64^{d}	32.79±0.59^{cd}	94.09±1.69^{cd}

Means with different letters in each row are significantly differs at p< 0.05; **AST:** Aspartate Aminotransferase; **ALT**: Alanine Aminotransferase; **ALP**: Alkaline Phosphatase.

Effect of Basil leaves on MDA and GPx in hepatotoxicity rats: Table 4 represents the results of lipid peroxidation as indicated by serum MDA level and activity of GPx in normal rats, untreated hepatotoxicity-rats and treated hepatotoxicity-rats with the oral administration of Baasil leaves. In comparison to normal rats, untreated hepatotoxicity rats (positive rats) have a significant increase (P<0.05) in serum levels of MDA and decrease in serum activities of GPx. However, oral administration of Basil leaves in (1% and 2%) encourages

a significant (P<0.05) decrease in serum MDA level and increase in the activity of GPx enzymes compared to the positive control group. The good result in serum concentration of MDA and activity of antioxidant enzymes was shown in the hepatotoxicity group that treated by 2% Basil leaves as compared to the other treated group.

Table 4: Effect of Basil leaves on MDA and GPx in hepatotoxicity rats.

Parameters / Groups	GPx (u/ml)	MDA (u/ml)
Negative group	87.50±1.19^{a}	36.30±0.35^{d}
Positive group	49.98±1.57^{e}	83.67±1.42^{a}
1 % Basil leaves	65.50±0.86^{d}	56.75±0.85^{b}
2 % Basil leaves	73.75±0.47^{c}	35.67±1.03^{d}

Means with different letters in each row are significantly differs at p< 0.05; **MDA:** Malondialdehyde and **GPx**: Glutathione Peroxidase.

Discussion

Regarding liver enzymes, rats injected with CCl_4 showed significant increase in the ALT and AST activities. These results agree with **Shah et al., (2015)** who attributed that CCl_4 causes injury of the membrane and so leakage of the cytosomal enzymes. That may be due to the reactive intermediate free radicals which are produced by CCl_4 bioactivation by cytochrome P450 **(Weber et al., 2003)**. Also, CCl_4 treatment causes an increase in the level of serum GGT, ALP and total, direct and indirect bilirubin. These results agree with **Li et al., (2015)**, the increase may be attributed to the increase in synthesis of GGT and ALP in case of the biliary pressure increase. Sweet basil (***Ocimum basilicum, L.***) is a widely cultivated annual herb with numerous pharmacological activities, including antioxidant, chemopreventive, anti-inflammatory, antimicrobial, anti-inflammatory, and immunomodulatory activity **(Teofilović et al., 2021)**. These are attributed to the abundance of phytochemicals identified in basil, including flavonoids, phenolic acids, rosmarinic acid and aromatic compounds, and essential oils eugenol, chavicol, linalool, and a-terpineol **(Purushothaman et al., 2018)**. In line with **(Bellassoued et al., 2021)** reported that in CCl_4 induced intoxicated rats, the plasma lipid levels cholesterol, triglycerides and LDL increased as compared to the control group, also a significant decrease in the HDL-Ch level. Treatment

with MECA alone did not cause any significant change in lipid profile. However, rats pre-treated with MECA exhibited a marked reversal of the serum lipid profile cholesterol, triglycerides and LDL compared to toxic control. Moreover, HDL-Ch level significantly increased as compared to the CCl4 group.As well **(Sakr et al., 2011)** aimed to investigate the effect of basil on Ccl4-induced hepatotoxicity and apoptotic in albino rats, reported that administration of CCl4 to rats caused significant increase in cholesterol, triglycerides and LDL compared with animals of control groups. Animals treated with both CCl4 and O. basilicum extract showed reduction in their sera level of cholesterol and triglycerides in comparison of those given CCl_4. The administration of OBL significantly decreased the serum ALT and AST, ALP, GGT, total, direct and indirect bilirubin close to normal control rats. Results from this study confirm with **Atangwho et al., (2014)** and **Meera et al., (2009)**. These results could be explained that OBL protects the hepatocytes from injuries and improves the function of liver **(Chiu et al., 2012)**.

Concerning antioxidants parameters, the increase of MDA in CCl_4 administered rats compared to normal rats may be attributed to the trichloromethyl radicals that resulted from CCl_4 metabolism. Those radicals stimulate the process of lipid peroxidation with the formation of byproducts such as MDA. **(Madubuike et al., 2015)** Also, in the present study, the hepatic antioxidant enzymes SOD and CAT and GPx were significantly decreased activities in CCl_4-intoxicated rats compared with control rats. These results partially match with **Tsai et al., (2009)**, decrease in enzyme activity may be attributed to the deactivation of their isoenzymes by oxidation of a cysteine residue near the active center. OBL compared with normal rats showed significant increase in liver MDA and significant decreases in liver SOD and GPx levels. These results partially agree with **Karaali et al., (2018)** who found that pretreatment with basils caused increase in serum ALT and AST and MDA in liver. OBL protected group demonstrated significant increases In GPx and SOD compared with CCl4 injected rats, while a significant decrease in MDA was observed.

Conclusion

Our study demonstrated that Basil leaves exert beneficial effects in mitigating carbon tetrachloride (CCl_4)-induced hepatotoxicity in rats. Supplementation with 2% Basil leaves significantly improved liver function markers (ALT, AST and ALP), lipid profiles (TC, TG, HDL- c, LDL-c and VLDL-c), and antioxidant status (increased GPX, decreased MDA), approaching values observed in the negative control group. The 2% Basil leaves consistently showed the most pronounced protective effects across all measured parameters, including improved body weight gain and feed efficiency ratio. These findings suggest a synergistic hepatoprotective and antioxidant potential of Senna leaves when used particularly at higher concentrations.

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التأثير الوقائي الكبدي لأوراق الريحان (*Ocimum basilicum L.*) على سمية الكبد الناجمة عن مركب كلوريد الكربون (CCl_4) في الفئران

الملخص العربي

صممت هذه الدراسة للتحقق من التأثيرات الوقائية للكبد لأوراق الريحان كمضادات الأكسدة (BL) على السمية الكبدية للفئران المحدث برابع كلوريد الكربون (CCl4) . كانت الحيوانات عبارة عن (٢٨) فارا ألبينو مقسمة إلى ٤ مجموعات. استُخدمت المجموعة ١ كعنصر تحكم وتلقت نظامًا غذائيًا أساسيًا يوميًا طوال الفترة. وتم حقن فئران المجموعة ٢) مجموعة (CCl4 بزيت الذرة (١:١) ١ مل/كجم، من وزن الفأر. فى اليوم الخامس عشر والثامن عشر والحادى والعشرين. المجموعة ٣ فئران تغذت على نظام غذائى مدعم ب(BL) 1%) 21/ يوم (ثم حقنت بجرعة CCl4 مثل المجموعة ٢. المجموعة ٤ أعطيت BL 2%) 21 /يوم (ثم حقنت ب :CCl4 مثل المجموعة ٢. تم تحديد مؤشرات التقييم البيولوجية (ووزن الجسم الأولي ووزن الجسم النهائي ومعدل الاستفادة من الغذاء ونسبة الزيادة في وزن الجسم) للفئران. كما تم فصل عينات الدم إلى سيرم الدم لتحديد وظائف الكبدALT) ، ASTو (ALP وعينة لتحديد مؤشرات مضادات الأكسدة MDA) و(GPx ، ومعدل الدهونTC) ، TG، HDL–c، LDL–c، .(VLDL–cوقد تحسنت ووزن الجسم الأولي ووزن الجسم النهائي ومعدل الاستفادة من الغذاء ونسبة الزيادة في وزن الجسم بشكل ملحوظ عن طريق تناول أوراق الريحان مقارنةً بالسموم الكبدية لدى الفئران. كشفت النتائج أنه في مجموعةCCl4 ، كانت هناك زيادات كبيرة في ALT و AST و ALPو TC و TG و TG و LDL–c و VLDL–c وMDA ، في حين أظهرت المعاملات الاخرى HDL–cو GPx انخفاضًا كبيرًا. وفي الوقت نفسه، أدى تناول أوراق الريحان إلى انخفاض كبير في جميع المؤشرات المذكورة سابقا وزيادة في HDL–c و .GPxولذلك، يمكن استنتاج أن هذا تناول أوراق الريحان كان له دور وقائي للكبد كمضاد للأكسدة.

الكلمات المفتاحية: أوراق الريحان، رابع كلوريد الكربون ، الكبد، مضادات الأكسدة، السمية، وقاية الكبد.