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Protective Effect of Phenolic Extract of *Urtica dioica* leaves against Carbon Tetra-Chloride Induced Hepatotoxicity in Male Rats

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ABSTRACT:
The present study concluded phenolic extract of *Urtica dioica* leaves the protecting effect against CCL_4 induced hepatotoxicity. The present study refered to a significant increase (P≥0.05) i serum levels of liver enzymes (ALP, ALT, AST), and bilirubin level in carbon tetrachloride-induced groups as compared with control group. The current study revealed the occurrence of significant decrease (P≤0.05) in the level of liver enzymes When administered by phenolic extract for (two and three months) compared with carbon tetrachloride group.
Highly significant (P≤0.05) with the concentration of the (500mg/kg) for phenolic extract, and no significant observed between phenolic extract for (two and three months) and before and afte induced by the carbon tetrachloride.
Histological section of liver male rat induced by carbon tetrachloride for two months showed inflammation and degeneration of hepatocytes, however, the histological section is shown chroni inflammation, severe congestion and haemorrhage, accumulation of fat, degeneration and necrosis, severe degeneration of hepatocytes, and appearance fibroblast for three months. Th histopathological changes of liver in male rats treated with both doses (250 and 500 mg/kg) of a phenolic extract of *Urtica dioica* leaves showed the normal architecture of hepatocytes.

KEYWORDS: *Urtica dioica*, phenolic, Hepatotoxicity, Carbon tetrachloride.

INTRODUCTION:

Hepatotoxicity:

Liver toxicity indicates to liver weakness in function or liver damage that is associated with an exposure of hepatotoxicants are chemicals that cause liver illness[1]. Hepatotoxicants ar compounds of clinical pertinence and may comprise over treatments of certain therapeutic drugs, industrial chemicals, natural chemicals like herbal medications and dietary complements[2].

Some medications might be liver damage when introduced as a medicinal substance for therapeutic[3]. Hepatotoxicity might be not just from direct poisonous of the essential compound (drug additionally from a receptive metabolite or from an immunologically-interceded reaction influencing hepatocytes[4,5].

The hepatotoxic reaction caused by an industry exposure relies on upon the grouping of the toxicant which might be either parent compound, lethal metabolite, or coupling of chemicals in vivo[6]

The liver serves as significant and vital part in various related capacities in the body, for example, detoxification and furthermore, capacity store of glycogen, which is additionally a middle fo the creation of several proteins i.e. plasma proteins[7].

The liver is a unique construction in the human body that have the ability to the regeneration of cells and re-increase[8]. This susceptibility is essential to compensate and protect hepatic cells fror any damage or deficiency in the performance of its functions[9].

The execution of the liver and its capacities are estimated in view of a few parameters, in particular, liver synthesis capacity, production of liver enzymes to determine the intact and preservatio of liver cells, the way that these compounds are discharged from the liver cells, and examination of the liver extraction ability of bilirubin creation[10].

For quite a while, in oldness drug, *U. dioica* has been utilised as a diuretic agent and to treat joint inflammation[11]. These days it is a crucial medicinal herb and consumed as a portion of th human eating regimen because of its substance of minerals, chlorophyll, amino acids, lecithin, carotenoids and vitamins[12]. Various substance constituents, for example, flavonoids, tannins an sterols have been disconnected from various parts of the plant[13].

Therefore, the current studies must be subjected to find natural alternatives from plants which have side effects almost non-existent and in addition to the referencing and the use of folk herba according to modern methods to extract useful such materials. Also, has to be considered to technology and modern science in the treatments where is the science and applications o nanotechnology leading in finding alternatives to treat diseases such as liver disease.

MATERIAL AND METHODS:

Material:

Chemicals and reagents:

CCL_4, methanol, chloroform, HCL, hematoxilin and eosin stain and paraffin wax were procured from [Merck Ltd., Coimbatore, Tamilnadu (India)].

Instrumentation:
The proposed work was carried out on Sexholate using to extract of phenolic compound, then evaporated on a rotary evaporation. Then, separation by funnel finally, weighted and stored in refrigerator until using. Microtome using the preparation of tissues section.

Selection of Solvents:
On the basis of solubility study, methanol was selected as the extract for phenolic compounds.

Preparation of Stock and working Solutions of methamol:
Methanol Stock Solution:
Concentrated solutions such as methanol and CCL_4, as well as ferric chloride, can be obtained from local laboratories.

Methanol Working Solution:
Prepare the methanol solution by adding 70ml of concentrated methanol to 30mL of distilled water to obtain a 70% methanol solution.

Ferric Chloride Working Solution:
Prepare ferric chloride solution by adding 1g of ferric chloride to 100ml of distilled water to obtain 1% ferric chloride solution.

CCL_4 Working Solution:
Prepare solution CCL_4 [(1ml/kg body weight) consolidated with olive oil [1:1][14]. The procedure of CCL_4 doses administration to male animal rats was orally for two and three months.

Preparation of phenolic extract:
Powder of Urtica dioica leaves was obtained from botany gardens of Baghdad University. The phenolic extract was prepared by adding 200mL of methanol alcohol to 20 g of powder, by usin Sexholate for 24 hours, The extract was evaporated on a rotary evaporation under vacuum at a temperature of 60Co until the solution reached to 10mL. Then, the solution was transferred to separation funnel and add 2N HCL was added gradually to get PH=2, then, washed with 10mL of chloroform three times. The solution was separated into two layers, the lower layer was drie which is phenolic, weighted and stored in a refrigerator until using[15].

Method:
Determination of phenolic compounds:
The triple drops of 1% ferric chloride (FeCl3) solution was combined with 2mL of each extract. The presentation of thick lavender colour with ferric ions designate the presence of Phenoli compounds[16].

Animals:
seventy male albino rats weighing (200-250g) and aged (10-17week). Animals were housed in the animal house of faculty of Science/University of Kufa under control condition; light 12 and 1 dark hours and temperature (24±2C°).

Animals divided into three groups as follows the control groups, carbon tetrachloride groups, and phenolic extract groups. Subdivided groups above depending on the concentration (250 an 500 mg/kg) of phenolic extract, for (two and three) month, add to dosage after and before induced by carbon tetrachloride (five animals per group).

Blood samples:
The blood was drawn through heart puncture by using disposable syringe (5mL), then left at room temperature for clotting, and then centrifugated at (3000 rpm) for (15) minutes, serum wa isolated and stored at deep freeze in Al- Sadar medical city in Al-Najaf Al-Ashraf province until using for measurements biomarkers and liver enzymes.

Liver enzymes and bilirubin:
The assessment of (Serum alanine aminotransferase (ALT), serum aspartate transaminase (AST), serum alkaline phosphatase (ALP) and bilirubin (Bb) rats Elisa kits provided by (Elabscience china) Sandwich immunoassay technique (enzyme-linked immunosorbent assay – automated microtiter plate), Elisa reader (Biokit ELX 800 reader, ELX50 washer/USA). Estimation was don according to the instruction of the supplier.

Histopathological assessment:
For Histopathological examination, The animals were sacrificed at end of the experiments by using an anaesthetizing xylene and ketamine (3:1mL) and liver from each animal was excised the immersed in neutral buffered formalin(10%) for 24 hr. Liver tissues were cleaned and embedded in paraffin, cut in 5µm sections, stained with the haematoxylin and eosin and examine microscopically.

Staining of histological sections:
Histological sections are stained by using acid Eosin Hematoxylin stain[17].

Analytical Discussion:
Results are represented as mean ± standard error (SE) and performed using one-way ANOVA by GraphPad Prism® software (GraphPad Software, Inc., La Jolla, CA, USA) L.S.D was P≤0.05 i study groups and data were compared between groups using *T*-test[18].

RESULTS:

Physiological Studies:
Effects of two doses (250 and 500 mg/kg) of *Urtica dioica* leaves phenolic extract in hepatotoxicity male rats induced by CCL_4.
Results of the table (1) showed a significant increase (P≤0.05) in liver enzyme levels (ALT, AST, ALP and bilirubin) in group induced by CCL_4 (120.03±1.5, 183.47±2.45, 197.32±2.21 3.08±0.16) respectively, as compared to the control groups (46.69±1.51, 55.8±1.09, 54.7±1.69, 0.66±0.03). Also, it showed the protective effects of both doses (250 and 500 mg/kg) before an after induced by CCL_4; additionally, there was a significant decrease (P≤0.05) in liver enzyme levels (ALT, AST, ALP and bilirubin), as compared with groups induced by CCL_4.

Table (1): showing the effects of two concentrations of phenolic extract of *Urtica dioica* leaves on liver function in hepatotoxicity male rats induced by CCL_4.

Parameters / Treated	Mean ± SE			
	ALT (ng/mL)	AST (ng/mL)	ALP (ng/mL)	Bilirubin (ug/mL)
CCL_4	120.03±1.15	183.47±2.45	97.32±2.21	3.08±0.16
250ph.b	90.50±1.80*	127.70±5.38*	83.90±1.21*	2.19±0.10*
250ph.a	86.40±1.63*	122.50±5.12*	78.40±1.46**	1.96±0.12*
500 ph.b	78.90±0.89**	116.20±8.38**	70.40±0.79**	1.68±0.08*
500ph.a	59.00±1.87***	91.50±1.16***	65.10±1.26**	1.30±0.05**
Control	46.69±1.51	55.80±1.09	54.70±1.69***	0.66±0.03***
L.S.D<0.05	**3.59**	**11.21**	**3.57**	**0.24**

(Ph: Phenolic extract, a: After administered CCL_4, b: Before administered CCL_4. Similar letters indicate significant absent, while the different letters indicate a significant presence compare induced groups vs control group. * P≤0.05, **P≤0.01, ***P≤0.001 compared treated groups vs induced group.)

Effect of duration of phenolic extract of *Urtica dioica* leaves (two and three months) in hepatotoxicity male rats induced by CCL_4:
The figures (1),(2),(3), and (4) showed a significant increase (P≤0.05) in the liver enzyme levels (ALT, AST, ALP and Bilirubin) in the CCL_4 induced group for both periods (two and thre months) as compared with control group**.**

These figures also explain the protective effects of the phenolic extract of *U.dioica* before and after induction for both periods; a significant decrease (P≤0.05) in the liver enzyme levels wa observed (ALT, AST, ALP and Bilirubin) before and after induced by CCL_4 for both periods.

The duration of extract treatment for three months after induction resulted in a significant increase (P≤0.05) alteration of all studied parameters, as compared with a period of two months (fo both before and after).

Figure (1): Effect of duration of phenolic extract of *U.dioica* leaves (two and three months) on ALT level in hepatotoxicity male rats induced by CCL_4.

Figure (2): Effect of duration of phenolic extract of *U.dioica* leaves (two and three months) on AST level in hepatotoxicity male rats induced by CCL_4.

Effect of interaction between doses and periods of phenolic extract of *Urtica dioica* leaves in hepatotoxicity male rats induced by CCL_4.

Figures (5,6,7 and 8) showed a significant increase (P≤0.05) in liver enzyme levels (ALT, AST, ALP and Bilirubin) in group induced by CCL_4 for both periods (two and three months (117±0.71, 176.2±2.06, 89.44±1.66, 2.74±0.09; 124±1.08, 189.8±1.28, 103±0.95, 3.48±0.21), respectively, as compared with group control (42.6±1.03, 52.4±1.03, 50.4±1.15, 0.6±0.03 46.2±1.53, 57.4±1.40, 59±1.18, 0.78±0.02). Also, this figures showed a significant increase (P≤0.05) with three months period, whereas it did not show any significant increase with the perio of two months.

Additionally, the results showed a significant decrease (P≤0.05) in liver enzyme levels (ALT, AST, ALP and Bilirubin) in treated groups, as compared with the induced group, as well as showed a significant decrease (P≤0.05), with a dose of (500mg/kg) for three months.

Figure (3): Effect of duration of phenolic extract of *U.dioica* leaves (two and three months) on ALP level in hepatotoxicity male rats induced by CCL_4.

Figure (4): Effect of duration of phenolic extract of *U.dioica* leaves (two and three months) on Bilirubin level in hepatotoxicity male rats induced by CCL_4.

Figure (5): Effect of interaction between two doses (250 and 500mg/kg) and duration (two and three month) of phenolic extract of *U.dioica* leaves on ALT level in hepatotoxicity male rat induced by CCL_4.

Figure (6): Effect of interaction between two doses (250 and 500mg/kg) and duration (two and three month) of phenolic extract of *U.dioica* leaves on ALT level in hepatotoxicity male rat induced by CCL_4.

Figure (7): Effect of interaction between two doses (250 and 500mg/kg) and duration (two and three month) of phenolic extract of *U.dioica* leaves on ALP level in hepatotoxicity male rat induced by CCL_4.

Figure (8): Effect of interaction between two doses (250 and 500mg/kg) and duration (two and three month) of phenolic extract of *U.dioica* leaves on Bilirubin level in hepatotoxicity male rat induced by CCL_4.

Histological Studies:

Histological section of male rat liver (control) for two and three months period showed normal histological structure Image (1) and (2) respectively. Histological sections of male rat live induced by CCL_4 for two months showed degeneration of hepatocytes (Img.3) and inflammation (Img.4); whereas the histological sections for three months showed chronic inflammatio (Img.5), severe congestion and hemorrhage (Img.6), accumulation of fat (Img.7), degeneration and necrosis (Img.8), severe degeneration of hepatocytes (Img.9), and appearance of som fibroblast (Img.10). The histopathological changes of liver sections treated with both doses (250 and 500 mg/kg) phenolic extract of *Urtica dioica* for two an three months (Img.11,12,13 and 14 showed the normal appearance of hepatocytes.

Image (1): Histological section of male rat liver (control) for two months period showed normal histological structure (HandE)400X.
Image (2): Histological section of male rat liver (control) for three months showed normal appearance (H&E)100X.
Image (3): Histological section of male rat liver induced by CCL_4 for two months showed degeneration of hepatocytes (H&E) 100X.
Image (4): Histological section of male rat liver induced by CCL_4 for two months showed inflammation (H&E)100X.
Image (5): Histological section of male rat liver induced by CCL_4 for three months showed chronic inflammation (H&E)100X.
Image (6): Histological section of male rat liver induced by CCL_4 for three months showed a severe congestion and haemorrhage (H&E) 400X.
Image (7): Histological section of male rat liver induced by CCL_4 for three months showed an accumulation of fat (defeating) (H&E)100X.
Image (8): Histological section of male rat liver induced by CCL_4 for three months showed degeneration and necrosis of hepatocytes (H&E) 400X.
Image (9): Histological section of male rat liver induced by CCL_4 for three months showed a Severe degeneration of hepatocytes (H&E)100X.
Image (10): Histological section of male rat liver induced by CCL_4 for three months showed the appearance of fibroblast (H&E) 100X.
Image (11): Histological section of male rat liver induced by 250mg/kg phenolic *U.dioica* for two months showed normal appearance (H&E)100X.
Image (12): Histological section of male rat liver induced by 250mg/kg phenolic *U.dioica* for three months showed normal appearance (H&E)100X.
Image (13): Histological section of male rat liver induced by 500mg/kg phenolic *U.dioica* for two months showed normal appearance (H&E)100X.
Image (14): Histological section of male rat liver induced by 500mg/kg phenolic *U.dioica* for three months showed normal appearance (H&E)100X.

DISCUSSION:

Liver Function Test:

ALT and AST enzyme levels when induced with CCL_4:

The results of a table (1) and figures (1,2,5 and 6) showed a significant increase in both ALT and AST level after orally induction by CCL_4. These findings are in agreement with the findings o (Wolf, 1999; Manna *et al*., 2006; Raja *et al*.,2007; 2012; Talwar *et al*., 2013)[19–22] who reported that hepatotoxic and hepatic damage induced by CCL_4 that resulted in increasing hepatic enzym marker of injury such as ALT and AST due to alteration of permeablilty of hepatic membrane and increase their levels in the circulation system reflected severe damage to the structure o integrity of the liver as well as hepatocytes necrosis[23].

ALT and AST enzyme levels when treated with phenolic extract of *Urtica dioica*:

The results of the table (1) and figures (1,2,5 and 6) indicate a significant decrease in AST and ALT levels after *U.dioica* treatment. These findings are in agreement with of the findings o Kataki *et al*., (2012)[24] who concluded that a considerable inhibition of hepatic necrosis and leakage of the intracellular enzyme by stabilising hepatocellular membrane.

Gilani and Janbaz, (1995); Bray *et al*., (2002); Olaleye *et al*., (2014)[25,26,27] reported that the extract of *U.dioica* contains (alkaloid, terpenoid, flavonoid, phenolic and mineral) that hav hepatoprotective ability led to free radical scavenger which promotes hepatoprotection and cellular membrane integrity of liver tissue membrane.

ALP enzyme and Bilirubin levels when treated with CCL_4:

The results of the table (1) and figures (3,4,7 and 8) showed a significant increase of ALP and bilirubin level after induction by CCL_4. These findings are in agreement with the findings o Krithika *et al*., (2009)[28] who mentioned that ALP is a marker of pathogenic alteration in biliary flow and CCL_4 induced elevation of ALP with a high level of serum bilirubin. Additionally Arhoghro *et al*., (2009)[29] noted that any increased in ALP level after CCL_4 treatment confirms hepatotoxicity of CCL_4 that resulted in a damage of cell membrane in liver tissue associated wit leakage of this enzyme into the circulation.

ALP enzyme and Bilirubin level when treated with phenolic extract of *Urtica dioica*:

The results of table (1) and figures (3,4,7 and 8) indicated a significant decrease of ALP and bilirubin level after orally administered with U. dioica, which are in agreement with the study o Joshi *et al*., (2015)[30] who reported that treatment with U. dioica leaves resulted in a significant decrease in ALP and bilirubin level, due to stabilizing hepatocellular membrane which led t stability of intracellular enzymes as well as the depletion of ALP activity with simultaneous suppression of raised bilirubin level that considered as an indicator for stability of bililary.

On the other hand, the high level of alkaline phosphatase in the serum is correlated its synthesis by cells lining bile canaliculi that usually in response to cholestasis and increased biliar pressure, increased ALP level which obtained after induction by CCL_4[31,32].

Effects of CCL_4 on liver tissues:

Histological sections of showed histological Histological section of figures (Image 3,4,5,6,7,8,9 and 10) showed histological degeneration, inflammation, chronic inflammation, sever congestio and haemorrhage, defeating, degeneration and necrosis sever degeneration, fibrosis after induction by CCL_4 for both periods in liver tissue after treatment with CCL_4 for two months. Thes changes are due to Eidi *et al*., (2012)[33] who reported that the acute hepatotoxic effect on a liver tissue after administrated of CCL_4 was confirmed histopatho logically by degeneratior inflammation and cell infiltration, congestion and sinusoidal dilation and with periportal fibrosis of portal triad. Many researchers confirmed that the histopathology of liver (degeneration) ar due to the reduction of antioxidant factor or due to decreasing the hepatocytes ability to produce antioxidant factors such as (superoxide dismutase), glutathione, reductase (G-SH) and exces production of (ROS) caused by toxic effect of CCL_4[34,35,36,37]. Moreover, another study stated that the multi-necrotic area surrounding and invaded by inflammation cell are due to an increas of (chemo-kinase) production which leads to aggregation of a mononuclear inflammatory cell and activation of sinusoidal cell in liver[13,38,39]. In our present study, the increased inflammator cells in liver tissue after CCL_4 administration it might due to an increase in the nitric synthase mRNA expression contribute to tissue damage but also UP-regulate the inflammation respons which inconsistent with Harbrecht *et al*., (1995) and Raj *et al*., (2013)[40,41]. And *Hsu et al*., (2009)[42]who reported that administrated CCL_4 resulted in acute fatty change, necrosis, foam degeneration. the fatty change may be caused by overproduction of (ROS) mainly (NO)[43].

Effect of phenolic extract of *Urtica dioica* on liver tissue:

Histological section of (Image 11,12,13 and 14) show the protective effect of the phenolic extract of *U. dioica* on the liver tissue and returning to its normal condition. The protective effects o *U. dioica* might be associated with the presence of flavone, phenolic and terpene compounds that might be reduced the effect of CCL_4 via the absent of fatty changes, necrosis and infiltration o lymphocytes after treatment with (20,40,80 mg/kg) *U.dioica*[44]. The results of Srinivasan *et al*., (2005)[45] suggested that the presence of phenolic compounds such as Ferulic acid have a hepati protective effect due to its ability to act as a free radical scavenger. The research studies rewired these was no toxicity demonstrated by *U.dioica* examined of the liver section and no patholog changes in mice treated with acetaminophen and *U.dioica* and this observation suggest regeneration of tissue after induction of damage[46,47,48]. A single dose of *U.dioica* (250mg/kg) was able t reduce the pathology of liver hepatocytes as a result of present bio-active compounds such as phenols that serves as free radical scavenger, which inhibits peroxidation process in cell membran and also inhibit formation of superoxide and hydroxyl radicals[49,50]. In the present study, the beneficial effect of *U. dioica* might be due to the activity of Beta-Carotene which attenuatin inflammation and fibrosis and the protective mechanism of Beta-Carotene as anti- fibrotic and anti-inflammatory that might be involved by enhancing the immunity and downregulation of ke cytokines.

CONCLUSION:

Administration of experimental male rats with carbon tetrachloride for two months represented a causative factor in inducing the hepatotoxicity.*Urtica dioica* have a protecting role agains carbon tetrachloride by its effect on free radical scavenger and activation of the antioxidant system. *Urtica dioica* are capable protecting the liver from the effect of carbon tetrachloride.

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