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Ameliorative Effect of Ethanolic Extract from *Cicer arietinum* Seeds towards CCl_4-Induced Liver Hepatotoxicity in Rats

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Abstract The ameliorative effect of the ethanolic extract from*Cicer arietinum* (Chickpea) seedstowards the CCl_4-induced hepatotoxicity in male Wistar rats through measuring certain biochemical parameters content in the liver were analyzed. The CCl_4-treated rats showed a significant decline in the studied the serum levels of high-density lipoprotein (HDL), albumin (A) as well as the hepatic levels of glutathione (GSH) and activities of catalase (CAT), superoxide dismutase (SOD) , glutathione reductase (GR), elevation in the levels of total lipids (TL), triglycerides (TG), total cholesterol (TC), low-density lipoproteins (LDL), globulin (G), total bilirubin (TBil) , alanine and aspartate aminotransferase and alkaline phosphatase (ALAT and ASAT, ALP) and the hepatic levels of malondialdehyde (MDA). In contrast, the administration of *C. arietinum* ethanol extract, notably improved all the studied parameters. This study showed that CCl_4 administration to Wistar rats, at a high dose level, could induce a hepatic injury in addition to certain metabolic alterations. The work was extended to investigate tissue histopathology. In conclusion, *C. arietinum* seeds ethanol extract, resulted in an attractive candidate for ameliorating of hepatotoxicity induced by CCl_4 through scavenging free radicals, improved liver functions, and normalizing the liver histopathological architecture.

Keywords *Cicer arietinum*, CCl_4, hepatoprotection, antioxidation, liver toxicity

Introduction

Liver is one of the most vital organs in the human body which is involved in the regulation of various biochemical functions [1]. It bears noting that the lack of proper management of liver disorders by regular medicinal system gives more relevance for the development of effective and safe naturally derived hepatoprotective drugs. A plethora of studies suggest that the consumption of fruits and vegetables rich in natural antioxidants reduce the risk of chronic hepatic diseases [2].

Carbon tetrachloride (CCl_4) is a potent environmental toxicant inducing severe hepatic damage *via* the generation of highly reactive free radicals. These radicals initiate lipid peroxidation by the covalent binding to phospholipid membranes which harm cellular permeability and finally leading to severe cellular damage [3-4]. The second damage of the liver occurs due to inflammatory responses which are initiated by Kupffer cells activation releasing proinflammatory mediators such as tumor necrosis factor-alpha (TNF-α). They stimulate other hepatic cells to attract and activate circulating inflammatory cells [5]. In this context, plants rich in natural antioxidants, in

particular, phenolic compounds have free radical scavenging ability with enhancement of the endogenous antioxidant enzymes *viz.* superoxide dismutase (SOD), catalase (CAT) as well as non-enzymatic antioxidants as reduced glutathione (GSH) [1 & 4]). Therefore, antioxidants rich plants could be potent hepatoprotective agents [6-7].

Natural products provide unlimited possibilities for brand spanking new drug leads due to the unrivaled availability of chemical diversity. On the alternative hand, using natural drug treatments has numerous advantages. One advantage is its extensive availability and easy in preparation. Plants can contain sugars, vitamins, unstable oils, glucosides, minerals, proteins, alkaloids, phenolic acids, esters, alcohols [8]. Most herbs may be used as medicine with the aid of using making decoctions. Traditional prescriptions normally include extracts and extracts and concentrated single active compound from plants [9]. Supporters of conventional herbal medicine experience that medicine is only in its herbal kingdom, which contains all the active ingredients preferably refined artificial drugs.

Among leguminous foods, chickpea (*Cicer arietinum* L.) is considered a basic food in many countries. They represent a source of carbohydrates, dietary proteins among other nutrients [10]. Several studies discussed secondary metabolites of chickpeas which include several phytochemical classes with a focus on phenolic compounds [11 - 12].

In the present investigation, we present the of hepatoprotective effect of *C. arietinum* seeds ethanolic extract towards carbon tetrachloride (CCl_4) induced injury in rats liver.

Materials and Methods

Materials

1-Plant Material

Seeds of the Egyptian chickpea cultivar 'Giza 1,' were purchased from the local market.

2- Chemicals Reagents:

All chemicals used were of high quality and analytical grade purchased from Sigma-Aldrich.

Preparation of the Extract

Seeds (250 g) of *C. arietinum* were grounded and then refluxed with 500 ml of 70 % ethanol for 6 hours, then filtered. The filtrate was concentrated under reduced pressure using a rotavapor to give a viscous gummy residue (22 g) to be used for investigations.

Assessment of Biological Activities

In vivo Hepatoprotective Activities

Animals

Male Wistar albino rats (120 to 150 g) were selected for this study. They were obtained from the Animal House, National Research Center, Egypt. All animals were kept in controlled environment of air and temperature with access of water and diet ad libitum. Anesthetic procedures and handling with animals complied with the ethical guidelines of Medical Ethical Committee of the National Research Centre in Egypt.

Experimental Design

18 male rats were used in this study. Animals were divided into 3 groups: **Group 1**, served as normal healthy control rats. **Group 2**, Rats were intraperitoneally injected with 500 microliters of CCl_4 diluted 1: 9 (v/v) in olive oil (0.1 ml) twice a week for six consecutive weeks. **Group 3**, Rats were intraperitoneally injected with CCl_4 (0.1 ml) followed by oral administration of *C. arietinum* ethanolic extract (200 mg/kg body weight).

Study of some Biochemical Parameters

Sample Preparations

Blood was collected from each animal by puncture of sublingual vein . Blood samples were collected into dry test tubes and then centrifuged at 3000 rpm in order to separate serum . The sera were kept at -20 ^{o} C for further

biochemical analysis. In order to collect the hepatic tissues, rats were immediately dissected . The liver was homogenized with 10% w/v ratio in ice-cold 50 mMTrisHCl buffer at pH 7.4 and then centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was collected and kept in deepfreeze at -20°C for further analyses.

Estimation of Serum Biochemical Parameters

In the serum of all the experimental groups, the levels of total lipids (TL), total cholesterol (TC), triglycerides (TG), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total proteins (TP), albumin (A), globulin (G), aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), alkaline phosphatase (ALP), total bilirubin (TBil) and direct bilirubin (DBil) were measured colorimetrically using Biodiagnostics kits (Dokki, Giza, Egypt).

Non-enzymatic and Enzymatic Antioxidant Assay

Lipid peroxide assay: The level of malondialdehyde (MDA) in the liver homogenate was assayed according to the described technique [13]. The principle of this method depends on the reaction of the liberated MDA after lipid peroxidation (LPO) of the cell membranes with thiobarbituric acid in acidic medium.

The concentrations of non-enzymatic (glutathione, GSH) as well as enzymatic (catalase, CAT, superoxide dismutase, SOD, glutathione reductase, GR) antioxidants were estimated in the homogenate of the liver of control and treated rats. The method by which GSH content was measured was based on the reaction of 5, 5'-Dithiobis-2-nitrobenzoic acid with GSH [14]. The CAT activity was estimated in accordance to the method described [15]. The SOD activity assessment was based on the ability of SOD to inhibit the reduction reaction of nitrobluetetrazolium dye mediated by phenazinemethosulphate [16]. The principle for measuring the GR activity was based on its ability to catalyze the reduction of glutathione (GSSG) [17].

Histopathological Study

Liver tissues were excised from sacrificed animals, individually weighed, and, from them, 5 µm thickness slices were cut, fixed in 10% paraformaldehyde, and embedded in paraffin wax blocks. Tissue sections of 5 µm thick were stained with hematoxylin and eosin (H&E).

Statistical analysis

Data were statistically analyzed by the aid of Statistical Package of the Social Sciences, SPSS version 23 (copyrighted by IBM SPSS software, USA). Data were expressed as a mean ± standard error of mean (SEM).

Results and Discussion

Hepatoprotective activities

Effect on serum biochemical parameters

The intraperitoneal administration of CCl_4 provoked significantly liver damage which was observed clearly by the elevated levels of ALT, AST, and ALP compared to the control group (Table 1). In the same manner, the raised levels of liver enzymes in the serum indicated their release from damaged hepatic cells associated with hepatic injury [18-19]. The oral administration of *C. arietinum* ethanolic extract exerted significant hepatoprotective activity by reduction of the aforementioned parameters compared to control (Table 1), this complies with results described previously [20-21]. This activity may be attributed to the characterized phenolics in the extract, *viz* hydroxybenzoic acid derivatives and biochanin A [22].

Similarly, the intraperitoneal injection of CCl_4 significantly decreased the level of serum albumin, indicating alteration of albumin synthesis in liver associated with hepatic intoxication and impairment of liver functions ([23]. This was accompanied with the elevated levels of globulins and consequently the reduction of albumin/globulins (A/G) ratio compared to the control group [24]. Remarkably, oral administration of the *C. arietinum* ethanolicextract, significantly restored the levels of serum albumin, and globulins to the control group indicating hepatoprotective activity with restoration of synthesis of albumin in liver.

Assessment of Lipid Profile

The induction of hepatotoxicity with CCl_4 significantly increased the level of serum total cholesterol by 125%, LDL and triglycerides with the significant decrease of serum level of HDL which was attributed to the alteration of lipoprotein metabolism in the liver [25] (Table 1). Remarkably, the oral administration of the *C. arietinum* ethanolic extract significantly restored the levels of serum total cholesterol, LDL, HDL, and triglycerides to the standard group indicating hepatoprotective activity with preservation of cellular integrity and antilipidemic effect. These results complying with previous reports on chickpeas sprout [26-27].

Table 1: Effect of oral administration of CCl_4 alone or with *C. arietinuethanolic* extract, on some biochemical parameters of male albino rats

Parameters	Experimental groups		
	Control	**CCl_4**	***C. arietinum* ethanolic extract + CCl_4**
TL ($mgdL^{-1}$)	512.04 ± 43.06	663.6 ± 42.3	621.2 ± 37.3
TC ($mgdL^{-1}$)	118.20 ± 12.97	230.8 ± 20.3	168.5 ± 11.2
TG ($mgdL^{-1}$)	104.40 ± 7.34	171.8 ± 14.5	125.6 ± 10.3
LDL-C ($mgdL^{-1}$)	61.20 ± 9.87	161.4 ± 16.7	114.7 ± 12.5
HDL-C ($mgdL^{-1}$)	36.60 ± 6.40	28.6 ± 3.5	30.1 ± 6.3
TP ($g\ dL^{-1}$)	6.68 ± 0.22	6.27 ± 0.3	6.35 ± 0.8
A ($g\ dL^{-1}$)	4.42 ± 0.13	3.2 ± 0.2	3.9 ± 0.06
G ($g\ dL^{-1}$)	2.46 ± 0.24	3.9 ± 0.1	3.1 ± 0.09
A/G ratio	1.72 ± 0.16	0.95 ± 0.07	1.5 ± 0.01
ASAT (UL^{-1})	33.02 ± 1.30	109.3 ± 25.9	78.2 ± 12.3
ALAT (UL^{-1})	25.60 ± 1.50	69.6 ± 9.7	48.3 ± 8.2
ALP (UL^{-1})	55.30 ± 3.84	79.2 ± 11.8	63.1 ± 6.5
TBil ($mg\ dL^{-1}$)	0.66±0.02	0.94 ± 0.05	0.82 ± 0.07
DBil ($mg\ d\ L^{-1}$)	0.11 ± 0.005	0.15 ± 0.009	0.13 ± 0.006

Data are represented as mean ± standard error.

Non-enzymatic and Enzymatic Antioxidant Assay

The effects of CCl_4 alone or with *C. arietinum* ethanolic extract administration on the degrees of hepatic MDA and GSH and the activities of endogenous cell reinforcement proteins were appeared in (Table 2).The *in vivo* antioxidant activity was determined *via* the assay of liver endogenous antioxidants *viz.* non-enzymatic (GSH) and enzymatic (CAT and SOD), and MDA as a marker of lipid peroxidation. The administration of CCl_4 significantly decreased the levels of endogenous antioxidants GSH, CAT and SOD with the significant increase of hepatic level of MDA. The oral administration of significantly restored the levels of them nearly to values of the control group indicating *in vivo* antioxidant activity. This complies with the data reported by Sri Ramachandra et al., [21] on aerial parts of chickpea extract. Consequently, the antioxidant and hepatoprotective activity could be associated with the phenolics present in *C. arietinum* according to Kinjo et al., [22].

Table 2: Effect of oral administration of CCL_4 alone or with various *C. arietinum* ethanolic extract, on the levels of some antioxidants of male albino rats

Parameters	Experimental groups		
	Control	**CCl_4**	***C. arietinuethanolic* extract + CCl_4**
MDA (nmol g^{-1} liver)	4.48 ± 0.11	9.8 ± 0.6	5.2 ± 0.7
GSH (mg g^{-1} liver)	40.04 ± 5.10	21.7 ± 7.8	29.5 ± 6.2
CAT (U g^{-1} liver)	104.3 ± 17.1	45.4 ± 9.7	93.4 ± 13.1
SOD (U g^{-1} liver)	9.56 ± 0.17	5.6 ± 0.9	6.9 ± 0.8
GR (U g^{-1} liver)	73.20 ± 2.71	34.8 ± 2.8	52.3 ± 6.5

Data are represented as mean ± standard error.

Histopathological investigation

Microscopic examinations of sections of liver from normal control rats show the normal architecture of hepatic lobules. The central veins lies at the center of the lobules surrounded by cords of hepatocytes. Between the strands of hepatocytes, the hepatic sinusoids are seen (Figure 1). Histopathological investigation of liver from rats administered with CCl_4 alone showing disruption of the liver tissue with lossof lobular arrangement, bridging fibrosis with collagenous septa formation expanded portal tract to central vein with mononuclear cells, vacuolar degeneration and necrosis of hepatocytes (Figure 2). Liver segments of rats treated with CCl_4 and *C. arietinum* ethanolic extract exhibiting a more normal architecture of liver with fewer hepatocytes showing fatty change with fewer number of hepatocytes surrounding the central vein had necrobiosis (Figure 3). The histopathological examination is complying with the previous reports on chickpeas [19-20].

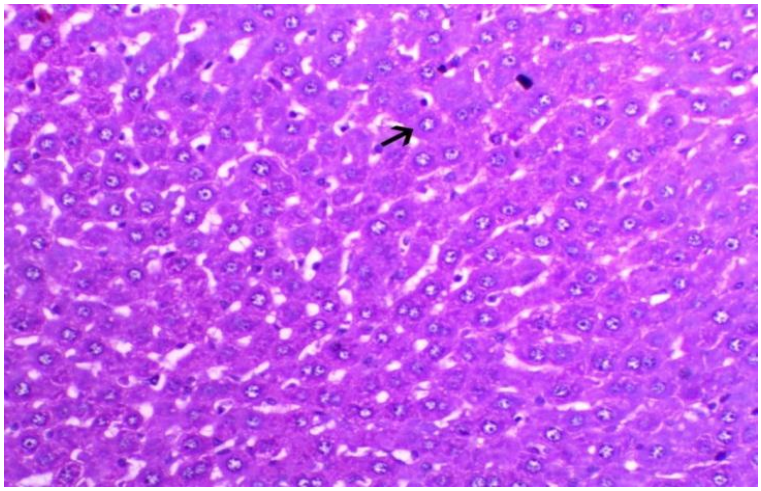

Figure 1: Photomicrograph of section in liver of control rat shows normal histological structure of hepatic lobules central vein, hepatocytes, blood sinusoids, and nuclei (H&E, ×400).

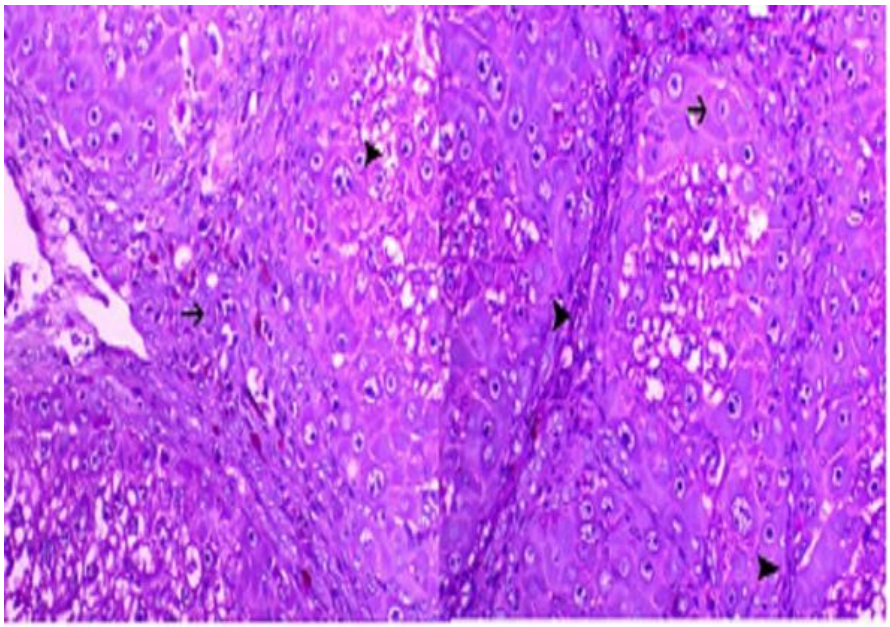

Figure 2: Photomicrograph of section in liver of rat administered with CCl_4 alone showing disruption of the liver tissue with loss of lobular arrangement, bridging fibrosis with collagenous septa formation expanded portal tract to central vein with mononuclear cells, vacuolar degeneration and necrosis of hepatocytes. Dilated and congested central vein was observed (arrowhead) and pyknotic nuclei (H&E, ×400)

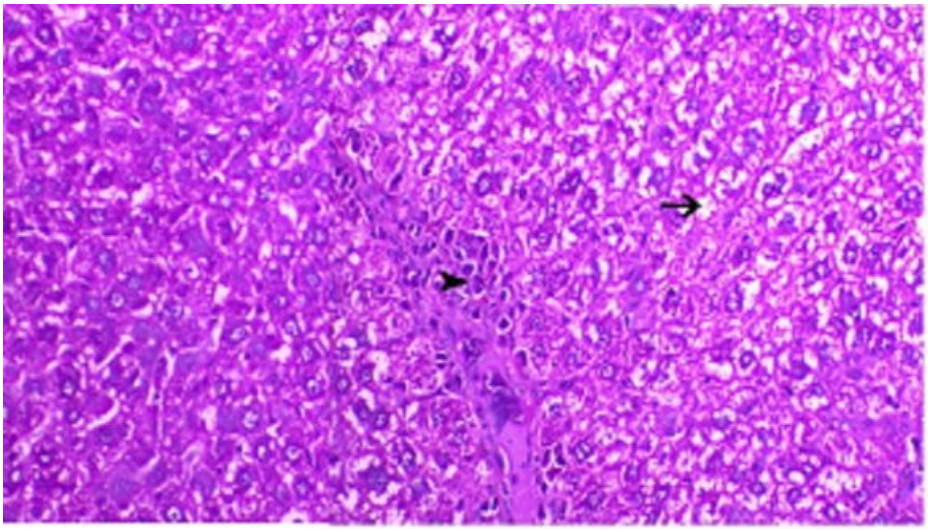

Figure 3: Photomicrograph of section in liver of rat administered with CCl_4 and C. arietinum ethanolic extract showing mild inflammatory cells infiltrations around central vein (arrow), vacuolar degeneration, and necrosis of hepatocytes (star). Binucleated and activated Kupffer cells were noticed (H&E, ×400).

In the present investigation, the biochemical findings were also confirmed by histpathological observations. The changes mostly include hepatocellular necrosis or apoptosis, fatty accumulation, inflammatory cells infiltration and other histological manifestations which were also consistent with the findings of other authors [28-29].

Conclusion

Our results revealed that no treatment-related toxicity was detected after the administration of chickpea (*Cicer arietinum*) ethanolic extract. This extract exhibited a strong hepatoprotective activity *in vivo* based on measurement of some biochemical parameters and oxidative status. The hepatoprotective activity was further confirmed from the histopathological examination. Therefore, further bio-guided studies are required to evaluate the individual contribution of chickpea active ingredients in hepatoprotective activity.

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