

Full Length Research Paper

Hepatoprotective and antioxidant effects of *Cichorium endivia* L. leaves extract against acetaminophen toxicity on rats

Mohamed Marzouk*, Amany A. Sayed and Amel M. Soliman

Zoology Department, Faculty of Science, Cairo University, Cairo, Egypt.

Accepted 28 November, 2011

The current study was carried out to elucidate the effect of hydroalcoholic extract of *Cichorium endivia* L. leaves (HCE) against acetaminophen-induced oxidative stress and hepatotoxicity in male rats. Oral administration of acetaminophen produced liver damage in rats as manifested by the significant increase in liver MDA and serum total lipids, total cholesterol, creatinine, total bilirubin and enzyme activities (AST, ALT and ALP). While a significant decrease in the levels of liver GSH, GST, SOD, CAT, serum total protein and albumin was recorded. Pre-treatment of rats with *C. endivia* leaves extract or silymarin for 21 days succeeded to modulate these observed abnormalities resulting from acetaminophen as indicated by the pronounced improvement of the investigated biochemical and antioxidant parameters. These results substantiate the potential hepatoprotective and antioxidant activity of *C. endivia* leaves extract. In addition, the hepatoprotective activity of HCE was found to be compatible with silymarin as a known hepatoprotective drug.

Keywords: Acetaminophen, *Cichorium endivia*, Hepatoprotective, rat, silymarin.

INTRODUCTION

The liver is a vital organ which regulates many important metabolic functions and is responsible for maintaining homeostasis of the body (Mayuren et al., 2010). During the metabolism, excessive free radicals are generated and may cause liver damage. Normally, the free radical level in the body is low because healthy organisms can neutralize, metabolize, or subtract the toxic effects by free radical scavengers such as superoxide dismutase and catalase (Fridovich, 1983). Acetaminophen (APAP) is one of the most widely used analgesic-antipyretic drugs and causes centrilobular liver necrosis, acute liver failure and death at high doses (Zhu et al., 2009). Plaa (2010) reported that no single parameter is representative of "liver function"; however, the substances released by damaged hepatic cells, enzymes appearing in the blood are the most useful as biomarkers of possible liver injury. Jodynis-Liebert et al. (2005) suggested that there is increasing interest with the antioxidants of natural origin because they could suppress the oxidative damage of a

tissue by stimulating the natural defense system. The reasons for the use of herbal medicine include the expensive cost of conventional drugs, adverse drug reactions, and their inefficacy (Aghel et al., 2007). The genus *Cichorium* (Asteraceae) is economically important because of its potent hepatoprotective, antioxidant and hypoglycemic effects (Mulabagal et al., 2009; Zeinab et al., 2011). Clinical research has confirmed the efficacy and safety of silymarin against hepatotoxicity caused by different agents (Eminzade et al., 2008). Therefore, the present study was undertaken to examine the possible hepatoprotective and antioxidant effects of *C. endivia* hydroalcoholic leaves extract on rats treated with APAP. The effect of silymarin, as a standard hepatoprotective reference drug, on the same parameters was considered.

MATERIALS AND METHODS

Chemicals

Acetaminophen (N-acetyl-p-aminophenol, paracetamol) (APAP) was purchased from Sigma-Aldrich (St. Louis, Mo, USA). Silymarin was purchased from SEDICO phar-

*Corresponding Author E-mail: Dr_marzouk@hotmail.com;
Tel: +202 33369426; Mobile +20106040606

maceutical Co., 6 October City, Egypt. All other chemicals were of analytical grade.

Plant material

The aerial parts of *Cichorium endivia* L. (Asteraceae) were collected from field in Giza governorate (Spring, 2009), Egypt and authenticated by the Herbarium of Botany Department, Faculty of Science, Cairo University. The plant leaves were cleaned, washed and dried in stream of hot air. The dried leaves were grounded to a fine powder with a laboratory electrical mill.

Preparation of leaves extract

500 g powdered dried leaves were soaked three days in ethanol-water (1:1, v/v) at 4°C then filtered using gauze and then fine filter paper. The obtained filtrate was concentrated under reduced pressure and dried by lyophilizer apparatus (LABCONCO lyophilizer, shell freeze system, USA). The dried extract obtained (57 g) was stored at dry place for next use in experiments (Sadeghi et al., 2008).

Preliminary phytochemical investigation

The preliminary phytochemical screening of the hydroalcoholic extract of *C. endivia* leaves (HCE) was carried out for qualitative identification of type phytoconstituents present (Claus, 1961; Khandelwal, 2000).

Determination of antioxidant activity (Scavenging Activity of DPPH Radical)

The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay was carried out for the evaluation of the antioxidant activity according to Brand et al. (1995). This assay measures the free radical scavenging capacity of the investigated extract. The following concentrations of *C. endivia* hydroalcoholic extract were prepared: 2.5, 3.75, 5, 10, 15, 20, and 25mg/ml. Similar concentrations of ascorbic acid (Vitamin C) were used as a positive control.

The decrease in absorbance (Abs) was measured at $\lambda = 517$ nm.

The radical scavenging activity was calculated from the equation:

% of radical scavenging activity = $[\text{Abs (control)} - \text{Abs (sample)}] / \text{Abs (control)} \times 100$.

Experimental animals

All animals handling procedures were accordance with

the guideline of the Animal Ethic Committee. Male albino rats *Rattus norvegicus* weighing 150-180 g were purchased from the animal house of National Research Center, Giza, Egypt. The animals were housed 6 per cage for 72 h before commencement of the experiments, for acclimatization, and were kept in controlled condition of temperature 18-20°C with a 12h light/ dark cycle. Rats were received a standard laboratory diet containing: Crushed wheat(46%),shredded barley(40%), fishmeal powder(9%),dried milk(3%),yeast(1%),minerals and vitamins(1%) and supplied with water *ad libitum* throughout the experimental periods.

Experimental protocol

Forty eight rats were randomly divided into six groups (8 animals/ group) as follows group 1 :served as untreated control group and received orally distilled water (2 ml/kg) ;Group 2: treated orally, by gastric gavage, with water dissolved leaves extract(HCE) in a dose of 500 mg/kg b.wt., for 21 days; group 3: rats orally administrated silymarin (SL)(150 mg/kg b.wt.), dissolved in water, for 21 days; Group 4: received a single oral dose of acetaminophen (APAP) (2 g/kg b.wt.), rats of this group were sacrificed 48 h after APAP administration; Group 5: rats administrated extract for 21 days and on day 21 received APAP; Group 6: rats administrated silymarin for 21 days and on day 21 received APAP. Rats of group 5 and 6 were sacrificed 48 h after APAP administration.

Blood collection and liver homogenate

At the end of the experimental periods, overnight fasting rats were sacrificed by cervical dislocation. Blood samples were collected in non-heparinized centrifuge tubes, allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 860 g for 20 min at 4 °C and then kept at -20 °C until used for the biochemical analysis. Animals were dissected and the liver sample was removed, washed using chilled saline solution, weighed, minced and homogenized in ice-cold sodium-potassium phosphate buffer (0.01 M, pH 7.4). The homogenate were centrifuged at 860 g for 20 min at 4°C, and the resultant supernatants were frozen at -20 °C for hepatic parameters assay.

Biochemical parameters assay

The following biochemical parameters were determined in the serum: total lipids (Knight et al., 1972), total cholesterol (Allain et al., 1974), total proteins (Tietz, 1994), albumin (Tietz, 1990), total bilirubin (Walters and Gerarde, 1970), creatinine (Tietz ,1986), aspartate transaminase (AST) and alanine transaminase (ALT) (Reitman and Frankel, 1957), alkaline phosphatase (ALP)

Table 1. Inhibition of DPPH by *Cichorium endivia* leaves extract

Concentrations of leaves extract and vitamin C (mg/ml)	% inhibition of DPPH by leaves extract	% inhibition of DPPH by vitamin C
2.5	73.75	74.92
3.75	71.40	73.08
5	67.73	69.40
10	56.19	61.20
15	40.64	56.19
20	30.94	54.01
25	12.88	44.82

DPPH: 2,2-Diphenyl-1-picrylhydrazyl.

Table 2. Serum AST, ALT and ALP of rats under normal and experimental conditions

Groups	Control	HCE	SL.	APAP	HCE+APAP	SL.+APAP
Parameters						
AST(U/ml)	198.68 ±15.66	214.94 ±11.67 (+8.18)*	205.38 ±21.14 (+3.37)*	395.40 ±2.02 ^a (+99.01)*	263.72 ±7.09 ^b (-33.30) [#]	261.62 ±8.03 ^b (-33.83) [#]
ALT(U/ml)	80.08 ±7.49	98.18 ±2.90 ^a (+22.60)*	96.78 ±4.02 (+20.85)*	202.68 ±16.78 ^a (+153.10)*	121.19 ±2.82 ^b (-40.21) [#]	126.56 ±4.40 ^b (-37.56) [#]
ALP(U/L)	124.12 ±8.83	109.400 ±6.44 (-11.86)*	94.45 ±3.19 ^a (-23.90)*	209.74 ±22.89 ^a (+68.98)*	112.37 ±4.75 ^b (-46.42) [#]	119.81 ±6.79 ^b (-42.88) [#]

Data are expressed as means ± SE of 8 rats.

C. endivia extract (HCE), silymarin (SL), acetaminophen (APAP), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP).

(*) % of change from control group.

(#) % of change from APAP group.

^a Significant at P<0.05 as compared to control group.

^b Significant at P<0.05 as compared to APAP group.

(Belfield and Goldberg, 1971). The following parameters were estimated in the liver tissue: lipid peroxidation in terms of malondialdehyde (MDA) (Ohkawa et al., 1979), reduced glutathione (GSH) (Beutler et al., 1963), glutathione-s-transferase (GST) (Habig et al., 1974), catalase (CAT) (Aebi, 1984) and superoxide dismutase (SOD) (Nishikimi et al., 1972).

Statistical analysis

Results were expressed as mean ± SE of eight animals. For comparison, One Way ANOVA test followed by Student's *t*-test was carried out and P <0.05 was considered as statistically significant. All computations were performed using SPSS version 15.0 software.

RESULTS

The phytochemical screening of *C. endivia* leaves extract

(HCE) showed different active constituents. These are glycosides, alkaloids, basic nitrogenous substances, flavonoids, saponins, tannins, unsaturated sterol and triterpenes. The HCE showed a high effective free radical scavenging in the DPPH assay. Table 1 shows that from concentration of 2.5 to 10 mg/ml of HCE, there is a well antioxidant activity. Meanwhile, the concentrations more than 10 mg/ml show a decrease in the antioxidant activity of HCE, as compared to ascorbic acid (Vitamin C, the reference standard). From the above results, it seems that HCE exhibited a noticeable antioxidant effect at low concentrations. Again, HCE and ascorbic acid show the greatest antioxidant effect at 2.5 mg/ml which represents 73.75% and 74.92% of DPPH inhibition, respectively.

As shown in tables 2-4, the results of this study revealed that the administration of *C. endivia* leaves extract alone or silymarin alone caused considerable toxic effects on some of the investigated parameters of rats as compared to their corresponding control rats.

Again, this study revealed that the administration of APAP caused a significant increase (P<0.05) in liver

Table 3. Serum biochemical parameters of rats under normal and experimental conditions

Groups	Control	HCE	SL.	APAP	HCE+APAP	SL.+APAP
Parameters						
T. protein (g/dl)	8.06±0.22	6.75±0.21 ^a (-16.25) [*]	6.41±0.30 ^a (-20.47) [*]	6.51±0.45 ^a (-19.23) [*]	7.36±0.23 (+13.06) [#]	7.61±0.28 (+16.90) [#]
Albumin (g/dl)	4.58±0.13	4.50±0.19 (-1.75) [*]	3.99±0.11 ^a (-12.88) [*]	3.64±0.08 ^a (-20.52) [*]	4.61±0.22 ^b (+26.65) [#]	4.82±0.13 ^b (+32.97) [#]
T. bilirubin (mg/dl)	1.96±0.18	1.78±0.05 (-9.18) [*]	1.93±0.09 (-1.53) [*]	3.62±0.15 ^a (+84.69) [*]	2.01±0.07 ^b (-44.48) [#]	2.05±0.12 ^b (-43.37) [#]
Creatinine (mg/dl)	1.99±0.09	2.00±0.03 (+0.50) [*]	1.67±0.02 ^a (-16.08) [*]	2.81±0.20 ^a (+41.21) [*]	2.05±0.04 ^b (-27.05) [#]	1.94±0.05 ^b (-30.96) [#]
T. lipids (g/dl)	0.64±0.02	0.63±0.02 (-1.56) [*]	0.91±0.02 ^a (+42.19) [*]	0.84±0.03 ^a (+31.25) [*]	0.78±0.02 (-7.14) [#]	0.77±0.02 ^b (-8.33) [#]
T.cholesterol (mg/dl)	151.56±3.55	120.16±4.98 ^a (-20.76) [*]	108.04±4.11 ^a (-28.76) [*]	190.79±6.90 ^a (+25.81) [*]	135.51±9.32 ^b (-28.97) [#]	191.70±11.90 (+0.48) [#]

Data are expressed as means ± SE of 8 rats.

C. endivia extract (HCE), silymarin (SL), acetaminophen (APAP).

(*) % of change from control group.

(#) % of change from APAP group.

^a Significant at P<0.05 as compared to control group.

^b Significant at P<0.05 as compared to APAP group.

Table 4. Liver antioxidant parameters of rats under normal and experimental conditions

Groups	Control	HCE	SL.	APAP	HCE+APAP	SL.+APAP
Parameters						
GSH (mmol/g tissue)	9.94±0.87	9.08±0.39 (-8.65) [*]	8.83±0.47 (-11.17) [*]	3.87±0.18 ^a (-66.85) [*]	9.66±0.50 ^b (+149.61) [#]	9.68±0.29 ^b (+150.13) [#]
GST (U/g tissue)	1.94±0.19	1.73±0.16 (-10.82) [*]	1.55±0.12 (-20.10) [*]	0.88±0.31 ^a (-54.64) [*]	1.66±0.20 (+88.64) [#]	1.87±0.08 ^b (+112.50) [#]
Catalase (U/g tissue)	2.72±0.44	2.07±0.53 (-23.99) [*]	2.23±0.64 (-18.01) [*]	1.04±0.19 ^a (-61.76) [*]	2.46±0.23 ^b (+136.54) [#]	1.98±0.18 ^b (+90.38) [#]
SOD (U/mg protein)	7.07±0.36	5.73±0.43 ^a (-18.95) [*]	8.17±0.45 (+15.56) [*]	3.32±0.37 ^a (-53.04) [*]	6.69±0.36 ^b (+101.51) [#]	6.90±0.49 ^b (+107.83) [#]
MDA (nmol/mg protein)	1.40±0.19	1.49±0.31 (+6.43) [*]	1.45±0.13 (+3.57) [*]	2.77±0.22 ^a (+97.86) [*]	1.67±0.18 ^b (-39.71) [#]	1.56±0.09 ^b (-43.68) [#]

Data are expressed as means ± SE of 8 rats.

C. endivia extract (HCE), silymarin (SL), acetaminophen (APAP).

(*) % of change from control group.

(#) % of change from APAP group.

^a Significant at P<0.05 as compared to control group.

^b Significant at P<0.05 as compared to APAP group.

MDA as well as total bilirubin, total lipids, total cholesterol and creatinine levels in serum. Meanwhile, a significant increase (P<0.05) in serum liver functions marker enzymes (AST,ALT and ALP) was recorded in APAP intoxicated rats as compared to untreated control group (Tables 2-4). On the other hand, a significant decrease(P<0.05) of liver GSH, GST, SOD, CAT as well as serum total protein and albumin was recorded in

APAP treated rats as compared to the untreated control group.

Pre-treatment with HCE or silymarin (SL), for 21 days prior to APAP treatment, reversed the changes caused by acetaminophen in most of the studied parameters. The results indicate that HCE and silymarin are effectively able to reduce the APAP-induced liver toxicity (Tables 2-4).

DISCUSSION

As regard to phytochemical constituents of *Cichorium endivia* leaves extract, the present study recorded the presence of several compounds in addition to high effective free radical scavenging activity in the DPPH assay. These findings suggested the antioxidant activity of such plant extract. In consonance with the present findings, Nandagopal and Kumari (2007) revealed the presence of alkaloids, flavonoids, triterpenoids, tannins and saponins in the root extract of *Cichorium intybus*. Again, Conforti et al. (2009) studied the hydroalcoholic extract of some edible plants and demonstrated their antioxidant and free radical scavenging activity.

The serum bilirubin, AST, ALT, and ALP are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction (Johnkennedy et al., 2010). In liver injury the transport function of hepatocytes is disturbed, resulting in the leakage of plasma membrane (Rajesh and Latha, 2004). The increase activities of AST, ALT, ALP and total bilirubin level in serum of APAP treated rats, observed in this study, indicates APAP-induced liver impairment. This was confirmed by an earlier study of Bhadauria and Nirala (2009) and Yuan et al. (2010) in which hepatic markers were reportedly elevated. The elevated activities of serum AST, ALT and ALP in APAP-induced liver injury indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Premila, 2005). The increase in total bilirubin level in the serum of APAP treated rats could be attributed to the increase in the rate of red blood corpuscles destruction and/ or damage of liver tissue (Hall, 2001). The present study suggested the ameliorative effect of HCE and silymarin on total serum bilirubin level and activity of AST, ALT and ALP of rats against APAP-induced changes. In consonance with our findings, Madani et al. (2008) disclosed the hepatoprotective activity of *Silybum marianum* and *Cichorium intybus* extract against thioacetamid in rats. Again, Sreelatha et al. (2009) suggested that *Coriandrum sativum* leaves extract and silymarin have protective effect against CCl₄ – induced hepatotoxicity in rats. The observed reduction in the levels of liver function enzymes as a result of HCE administration might probably be due in part to the presence of antioxidant compounds which aided in reducing the liver injury induced by APAP. This effect was possibly by the ability of HCE in reducing the oxidative stress and enhancement of the endogenous antioxidant defense status (Nayeemunnisa, 2009). Again, this is an indication of the stabilization of plasma membrane (Chin et al., 2009).

According to Lyanda et al. (2010), estimation of total protein level in serum is an important way to assess acetaminophen-induced hepatic damage. Protein metabolism is a major project of liver and a healthy functioning liver is required for the synthesis of the serum proteins (Kanchana and Sadiq, 2011). The present

results demonstrated that serum total protein and albumin of rats were significantly decreased in response to APAP administration. The decreased level of serum total protein is concomitant with those observed by Thirunavukkarasu and Sakthisekaran (2003) and may be attributed to an increase in amino acid deamination (Varely, 1987) and /or significant fall in protein synthesis (Kanchana and Sadiq, 2011), which could be due to the peroxidative damage of liver (Bharathi et al., 2011). The present study disclosed the protective effect of HCE and silymarin against the APAP-induced damage, since they ameliorated the changes caused by APAP treatment. Similarly, Sadeghi et al. (2008) reported that total protein and albumin concentrations were decreased in CCl₄ treated animals, in comparison with the control group and they restored almost to near the normal values in groups which were treated with *Cichorium intybus* leaves extract.

Creatinine is an important biochemical parameter for diagnosis of renal impairment (Simeon et al., 1995). It was observed from the present study that administration of APAP caused a pronounced increase in serum creatinine level when compared with the control. This was in agreement with the work of Ojiako and Nwanjo (2006) in which administration of drugs that inhibit prostaglandin synthesis like paracetamol, ibuprofen exacerbate renal failure. The present work suggests the nephroprotective ability of HCE, since pre-treatment of rats with HCE caused a significant reduction in creatinine level which was elevated due to APAP. In accordance with the present study, Adeneye and Benebo (2008) reported that elevation of serum creatinine was significantly attenuated by *Phyllanthus amarus* treatment. The nephroprotective ability of HCE recorded in the present study may be due to the presence of antioxidant compounds, as flavonoids and triterpenes as well as its free radicals scavenging effects (Annie et al., 2005).

A significant decrease in serum total cholesterol level was recorded in rats administrated HCE or silymarin, singly. Sobolova et al. (2006) reported that silymarin and its polyphenolic fraction reduced cholesterol absorption in rats fed on high cholesterol diet. The cholesterol lowering property of HCE may be attributed to the presence of alkaloid, flavonoid and glycoside components (Vinuthan et al., 2007) and its ability to increase of lecithin-cholesterol acyl transferase activity (Shigemastu et al., 2001).

Total lipids and total cholesterol levels were increased significantly in the serum of APAP intoxicated rats group. The possible explanation of the observed hyperlipidemia might reflect the impairment of liver cells to metabolize lipids or lipid peroxidation (Berne and Levy, 1998). The increase in serum lipids may attributed to the increased hepatic synthesis and/or diminished hepatic degradation; reduced lipoprotein lipase activity plays a role in the increment of lipids (Mathur et al., 2005). Pre-treatment rats, for 21 days, with HCE or silymarin prevented the acetaminophen-induced rise in serum total lipid and total

cholesterol. These findings demonstrated their protective action on hepatic injury induced by APAP. Similarly, treatment with silymarin and *Calotropis procera* flower extract (Ramachandra Setty et al., 2007), and with silymarin and *Hippophae rhamnoides* (Hsu et al., 2009) significantly lowered serum cholesterol content of animals received paracetamol or CCl₄, respectively.

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT and GST. Glutathione (GSH) is one of most abundant tripeptide antioxidant present in liver. It has been suggested this compound protect thiol groups of protein from oxidation by free radicals (Biswas et al., 2010). When the balance between ROS production and antioxidant defense is lost, oxidative stress results, which through a series of events deregulates the cellular functions leading to various pathological conditions (Kuriakose and Kurup, 2010). Lipid peroxidation is an autocatalytic process, which is a common consequence of cell death. This process cause oxidative tissue damage in toxicity of xenobiotic (Gupta et al., 2004). In the present study, the rise in the liver MDA level and the decline in the level of liver GSH, GST, CAT and SOD in APAP-treated rats suggests enhanced lipid peroxidation during tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals. Kurata et al. (1993) reported that MDA is one of the endproducts in the lipid peroxidation process. Pre-treatment with HCE or silymarin, for 21 days, significantly reversed all of these changes induced by APAP. The possible mechanism by which HCE exhibited significant protection against APAP-induced hepatotoxicity may be due to the active constituents present in various ingredients like glycosides, basic nitrogenous substances, flavonoids, alkaloids, unsaturated sterols, saponins, tannins and triterpenes as well as its free radical scavenging activity. In consonance with our results, DeFeudis et al. (2003) and Mayuren et al. (2010) suggested the antioxidant properties of flavonoids, alkaloids and triterpenoids in liver injury.

In conclusion, the results of this study demonstrate that HCE has a potent hepatoprotective action upon acetaminophen-induced hepatic damage in rats. Our results show that the hepatoprotective effects of HCE may be due to its antioxidant and free radical scavenging properties. This protective property was found to be comparable to that of silymarin. However, further investigations are required to identify, isolate, characterize and evaluate the active principal responsible for such hepatoprotective activity.

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