HEPATOPROTECTIVE EFFECTS OF AQUEOUS PURSLANE (REJLAH) JUICE IN CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY IN MALE RATS

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Abstract:

Purslane (Portulaca oleracea L.) in Arabic (Rejlah) is a member of Portulacaceae family and used in folk medicine as antidiabetic and hypotensive plant. The currently study was designed to assess hepatoprotective effects of aqueous purslane juice against CCl₄-induced hepatotoxicity in rats. Thirty five male albino rats divided into 5 groups, 7 rats each, were used. Group I served as normal group, groups II, III, IV and V were injected subcutaneously with CCL₄ (2 ml/kg) to induce liver toxicity. Group II served as positive group, groups III, IV and V were given orally aqueous purslane juice at doses of 5, 10 and 15 ml/kg of b. wt, respectively. Serum (lipid profile), kidney functions, (liver enzyme) activities; and liver MDA concentration and GSH, SOD and CAT activity were determined. Histo-pathological examination of the liver was performed to determine the extent of cells damage induced by CCl₄. Results showed that untreated hepatotoxicity rats have significant increase in serum TL, TG, TC, total and direct bilirubin, BUN and creatinine, GGT, AST, ALT, ALP; and liver MDA concentration, while decreased TP, albumin levels, liver activities of GSH, SOD and CAT, compared to normal rats. Aqueous purslane juice significantly improved all of the above parameters which were getting better with increasing the dose of juice. Histopathological assay showed multifocal hepatic cell necrosis, pericentral hepatic cell necrosis and focal area of mononuclear cells infiltration in positive rats and treated rats with 5 and 10 ml/kg b. wt of aqueous purslane juice, respectively. Congested central vein was showed in some livers and apparently normally central veins and hepatocytes in other livers of treated rats with 15 ml/kg b. wt of aqueous purslane juice. In conclusion, purslane juice has hepatoprotective effect against hepatotoxicity in rats. These results affirm that the traditional use of purslane plant for the protection of hepatotoxicity may be beneficial for patients with liver diseases and hence it is worth studying on humans.
Keywords: CCl₄, liver toxicity, liver and kidneys functions, antioxidant enzymes.

Introduction:

Purslane (*Portulaca oleracea* L.) in Arabic known as (Rejlah) is a member of Portulacaceae family and widespread as a type of weed (Oiu *et al.*, 2000). Fresh green purslane is eaten as cooked vegetable and/or salad all around the world and might be used medicinally for a variety of cases including mastitis, aching urination, headache, stomach pain and intestinal inflammation (Leung and Foster, 1996). Folk medicine in some parts of China use it as antidiabetic and hypotensive plant (Meng and Wu, 2008). Purslane contains many of active constituents as coumarins, flavonoids, alkaloids, saponins (Sakai *et al.*, 1996), nutrients including minerals and vitamins (Mohammad *et al.*, 2004), fatty acids (Xin *et al.*, 2008), glutamic acid, aspartic acid and glutathione (Al-Howiriny, 2008). Several study show purslane exhibits skeletal muscle relaxant effect (Parry *et al.*, 1993), analgesic and antioxidant activity (Xiaoling, 1999), anti-inflammatory effects (Chan *et al.*, 2000), antifungal activity (Oh *et al.*, 2000), antidiabetic (Gong *et al.*, 2009) and wound healing properties (Rashed *et al.*, 2003). It also contains active component for the treatment of parasitic infectious diseases such as trypanosomiasis and leishmaniasis (Costa *et al.*, 2007).

Liver is the largest organ in human body and regulates several important functions including metabolism, detoxification of hepatotoxicants which cause hepatic injury during metabolic reaction. The manner for development of liver disease includes all cell types in the liver via death and regeneration processes and progress to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (Giannelli *et al.*, 2003). Oxidative stress has been considered a major contributor to the development of various types of hepatic disorders (Albano, 2008 and Aram *et al.*, 2009) and play a major role in the initiation and/or progression of carcinogenesis by inducing oxidative stress in liver cells (Gulcin, 2006). Carbon tetrachloride (CCl₄) is used for induction of liver injury (Kim *et al.*, 2010) and affects several organs of the body such as brain, hearts, lungs, kidneys and testes in experimental study, this means that liver is not only the target organ of CCl₄ (Ozturk *et al.*, 2003). CCl₄ toxicity results from bioactivation of CCl₄ into trichloromethyl free radical by
cytochrome P450 system in liver microsomes and causes lipid peroxidation of membranes that leads to liver injury (Cui et al., 2009).

The present investigation was designed to evaluate the potential hepatoprotective effects of aqueous purslane juice at different doses against CCl₄-induced hepatotoxicity in rat models.

**Materials and Methods:**

**Materials:**

**Plant:**

Fresh purslane was collected from village farms, Qalyubia, Egypt. It was sorting at the herbal plants center in the Ministry of Agriculture, Giza, Egypt.

**Rats:**

Male adult albino rats weighing 200 ± 10 g were purchased from Faculty of Veterinary Medicine, Cairo University, Egypt. They were acclimatized at the animal house conditions (25°C and 55% humidity with 12-hr light/12-hr dark schedule) for one week before starting experiment and fed with a basal diet and water *ad libitum*.

**Chemicals and Kits:**

Carbon tetrachloride (CCl₄), formalin and diethyl ether and other chemicals were purchased from El-Gomhorya Company for Chemical and Pharmaceutical, Cairo, Egypt. Kits for biochemical analysis of total lipids (TL), triglyceride (TG), total cholesterol (TC), gamma-glutamyl transferase (GGT), aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphates (ALP), total bilirubin (TB), direct bilirubin (DB), total protein (TP), albumin (Alb), malondialdehyde (MDA) reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), blood urea nitrogen (BUN) and creatinine (Cr) were purchased from the Gamma Trade Company for Pharmaceutical and Chemicals, Dokki, Egypt.

**Methods:**

**Preparation of aqueous purslane juice:**

Juice of purslane plant was prepared as described by (Mohamed et al., 2011) with some modifications. The fresh purslane plant was cleared
from any blemishes or obvious defects and cleaned with tap water from
dust and other foreign materials. Aqueous juice was prepared by mashing
the purslane plant and left it for about 24 h at the refrigerator. Then, the
resulting crude extract was filtered. The filtrate juice was kept at the
refrigerator till uses.

**Preparation of basal diet:**

The basal diet (AIN-93M) was prepared as reported by (Reeves *et al.*, 1993). It consists of casein 14%, soybean oil 4%, Choline chloride
0.20%, vitamin mixture 1.0%, mineral mixture 3.5%, fibers 5%, L-
Cystine 0.18%, sucrose 10% and the reminder was corn starch.

**Induction of hepatotoxicity:**

Induction of liver toxicity in rats encouraged by the subcutaneous
injection of CCL₄ at a dose of 2 ml/kg according to the method described
by Sundaresan and Subramanian (2003).

**Experimental groups:**

Thirty five male albino rats were divided randomly into 5 groups
comprising seven rats in each group and fed the basal diet with water *ad
libitum* during experimental period (4 weeks). Animals were grouped as
follows:

Group I: Rats was injected subcutaneous with paraffin oil only and
served as normal control group.

Group II: Rats was injected subcutaneous with CCL₄ (2 ml/kg b. wt)
and served as positive control group.

Group III: Rats was injected subcutaneous with CCL₄ (2 ml/kg b. wt)
and given orally aqueous purslane juice at a dose of 5 ml/kg b. wt.

Group IV: Rats was injected subcutaneous with CCL₄ (2 ml/kg b. wt)
and given orally aqueous purslane juice at a dose of 10 ml/kg b. wt.

Group V: Rats was injected subcutaneous with CCL₄ (2 ml/kg b. wt)
and given orally aqueous purslane juice at a dose of 15 ml/kg b. wt.

At the end of the experimental period (4 weeks), all animals were fasted
for 12-hr. (except of water) and then sacrificed under light diethyl either.
Blood samples were collected by cardiac puncture in centrifuge tubes for
serum separation. Blood samples were centrifuged for 15 minutes at 3000
rpm for serum separation. Then, serum was carefully pick out, transfers into dry clean test tubes and frozen at -10ºC until use for biochemical analysis. Liver of all animals were cut into two parts one was immediately prepared to determine biochemical parameters and the other was immersed in neutral buffered formalin 10% for histopathology examination.

**Biochemical analysis:**

Serum concentrations of total lipids (TL) were determined colorimetric using spectrophotometer apparatus adjust at 520 nm as described by Bachorik et al., (1996). Serum triglyceride (TG) and total cholesterol (TC) levels and serum activity of gamma-glutamyl transferase (GGT) were determined using enzymatic methods as described by Fossati and Principe, (1989) and Artiss and Zak (1997) and Tietz, (1995). Serum activity of aspartate transaminase (AST) and alanine aminotransferase (ALT) were assayed colorimetric by the method of Reitman and Frankel, (1957). Alkaline phosphates (ALP) was determined by the method of king and king, (1954). Assay of serum total protein (TP) levels were colorimetric as described by Young (1995). Total bilirubin (TB) and direct bilirubin (DB) were determined according to the method by Young (2000). Serum concentrations of alkaline phosphates (ALP) were assay as described by Tietz (1991). Concentrations of blood urea nitrogen (BUN) and creatinine (Cr) were determined using colorimetric kinetic as described by Waiker and Bonventre, (2008).

**Liver Tissue homogenate preparation:**

Part of the liver of all animals were cut into small pieces and immediately homogenized in 5-10 ml ice-cold medium containing buffer (50 mm potassium phosphate PH 7.5 containing 2mM EDTA) per gram tissue using tissue homogenizer (Sonicator, model 4710, Cole-Parmer Instrument Company, USA). The homogenates tissues were centrifuged at 4000 rpm for 15 min at 4ºC. Then, supernatant was carefully separate for the determinations of MDA, GSH, SOD and CAT according to Montgomery and Dymock (1961).

**Histopathological examinations:**

The other part of animal livers was immersed in neutral buffered formalin (10%) for 24 h. The fixed tissues were processed routinely, embedded in
paraffin, sectioned, deparaffinized and rehydrated using the standard techniques. The extent of CCl₄-induced necrosis was evaluated by assessing the morphological changes in the liver sections stained with hematoxylin and eosin (H and E) (Bancroft and Gamble 2002).

**Statistical analysis:**

Results were expressed as means ± S.D. Statistical evaluation was done using one-way analysis of variance (ANOVA) (Snedecor and Cochran, 1981). Values of P < 0.05 were considered significant.

**Results and Discussion:**

The results recorded in Table 1 showed that injection of CCl₄ caused significant increase in serum levels of TL, TG and TC (464.26±0.77, 164.74 ± 1.42 and 132.53 ± 1.50 mg/dl) compared to that of the normal control rats (318.25 ± 1.38, 95.43 ± 1.29 and 74.84 ± 0.59 mg/dl), respectively. However, administration of aqueous purslane juice at the three different doses to hepatotoxicity rats significantly lower the elevations in serum levels of TL, TG and TC compared to that of untreated positive rats.

Table (1): Effect of different dosages from aqueous purslane juice on serum levels of TL, TG and TC in CCl₄ induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters as Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TL (mg/dl)</td>
</tr>
<tr>
<td>Normal control rats</td>
<td>318.25±1.38^e</td>
</tr>
<tr>
<td>Positive control rats (CCl₄ treated)</td>
<td>464.26±0.77^a</td>
</tr>
<tr>
<td>Aqueous purslane juice (5 ml / kg b. wt) + CCl₄</td>
<td>404.15±0.57^b</td>
</tr>
<tr>
<td>Aqueous purslane juice (10 ml / kg b. wt) + CCl₄</td>
<td>355.00±1.57^c</td>
</tr>
<tr>
<td>Aqueous purslane juice (15 ml / kg b. wt) + CCl₄</td>
<td>321.18±1.15^d</td>
</tr>
</tbody>
</table>
Means with different letters in each column are significantly differs at p≤ 0.05.

SD: Standard Division of mean

The serum activities of GGT, AST, ALT and ALP as liver function are given in Table 2. It was found a significant increase (p < 0.05) in serum GGT, AST, ALT and ALP activities of CCl₄ treated rats compared to that of the normal rats. However a significant decrease (p < 0.05) was observed in the respective serum activities of hepatotoxicity rats given orally aqueous purslane juice compared with that of CCl₄ treated group only (positive rats).

Table 3 showed effect of CCl₄ on developed hepatocellular damage as evident from a significant lower in serum levels of TP and Alb (47.36±1.30 g/dl and 23.74±0.97 g/l), respectively and higher in total and direct bilirubin (2.19 ± 0.30 and 1.96 ± 0.08 mg/dl), respectively compared to that of the normal control rats. In contrast, treated rats with aqueous purslane juice have significantly restoration of the altered biochemical parameters at the entire dose levels (5, 10 and 15 mg/kg b.wt.) compared to that of untreated positive group. Data also showed that treated rats with the higher dose of aqueous purslane juice have no significant changes in serum levels total and direct bilirubin compared to that of normal rats.

**Table (2): Effect of different dosages from aqueous purslane juice on serum levels of GGT, AST, ALT and ALP in CCl₄ induced hepatotoxicity rats**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters as Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GGT (U/L)</td>
</tr>
<tr>
<td>Normal control rats</td>
<td>23.16±1.04</td>
</tr>
<tr>
<td>Positive control rats (CCl₄ treated)</td>
<td>89.96±0.54</td>
</tr>
<tr>
<td>Aqueous purslane juice</td>
<td>69.44±2.03</td>
</tr>
</tbody>
</table>
Means with different letters in each column are significantly differs at p≤ 0.05.
SD: Standard Division of mean

Table (3): Effect of different dosages from aqueous purslane juice on serum levels of TP, TB, DB and Alb in CCl₄ induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters as Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP (g/dl)</td>
</tr>
<tr>
<td>Normal control rats</td>
<td>65.86±1.02 b</td>
</tr>
<tr>
<td>Positive control rats (CCl₄ treated)</td>
<td>47.36±1.30 c</td>
</tr>
<tr>
<td>Aqueous purslane juice (5 ml/ kg b. wt) + CCl₄</td>
<td>55.34±0.87 d</td>
</tr>
<tr>
<td>Aqueous purslane juice (10 ml/ kg b. wt) + CCl₄</td>
<td>59.71±0.78 c</td>
</tr>
<tr>
<td>Aqueous purslane juice (15 ml/ kg b. wt) + CCl₄</td>
<td>67.84±1.17a</td>
</tr>
</tbody>
</table>

Means with different letters in each column are significantly differs at p≤ 0.05.
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Table 4 revealed that injurious liver tissues due to CCl₄ injection characterized by the significantly higher concentrations of liver MDA (9.38 ± 0.47 nmol/ mg tissues) and lower GSH, SOD and CAT activities (26.40±0.84, 22.66±1.28 and 27.73 ± 0.72 µ/mg tissues, respectively) compared to that of the normal control rats. However, orally administration of aqueous purslane juice at the different doses with CCl₄
injection to rats significantly decreases liver concentrations of MDA, while increases towards normal rats of GSH, SOD and CAT activities compared to that of untreated positive rats.

Table (4): Effect of different dosages from aqueous purslane juice on liver concentrations of MDA, GSH, SOD and CAT in CCl₄ induced hepatotoxicity rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>MDA (nmol/mg tissues)</th>
<th>GSH (µ/mg tissues)</th>
<th>SOD (µ/mg tissues)</th>
<th>CAT (µ/mg tissues)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control rats</td>
<td>2.19±0.24d</td>
<td>52.51±1.75b</td>
<td>58.36±0.93a</td>
<td>68.13±1.21a</td>
</tr>
<tr>
<td>Positive control rats (CCl₄ treated)</td>
<td>9.38±0.47a</td>
<td>26.40±0.84c</td>
<td>22.66±1.28d</td>
<td>27.73±0.72c</td>
</tr>
<tr>
<td>Aqueous purslane juice (5ml / kg b. wt) + CCl₄</td>
<td>7.26±0.36b</td>
<td>38.76±0.69d</td>
<td>33.91±0.82c</td>
<td>48.01±1.20d</td>
</tr>
<tr>
<td>Aqueous purslane juice (10ml/kg b. wt) + CCl₄</td>
<td>3.56±0.25c</td>
<td>46.36±0.98c</td>
<td>46.47±0.89b</td>
<td>57.08±1.33c</td>
</tr>
<tr>
<td>Aqueous purslane juice (15 ml/kg b. wt) + CCl₄</td>
<td>2.33±0.31d</td>
<td>57.71±0.98a</td>
<td>58.06±0.69a</td>
<td>64.90±1.96b</td>
</tr>
</tbody>
</table>

Means with different letters in each column are significantly differs at p≤ 0.05.

SD: Standard Division of mean

The kidney functions biomarkers of rats in all groups are presented in Figures 1 and 2. BUN and Cr levels were increased significantly (p < 0.05) in the CCl₄ treated group only compared to that of the normal control rats (Figs 1 and 2, respectively). However, values of BUN and Cr were significantly corrected towards the control values in rats intoxicated with CCl₄ and treated with aqueous purslane juice at the three different doses.
Fig. (1): Effect of aqueous purslane juice on BUN level in CCl₄ induced hepatotoxicity rats

Fig. (2): Effect of aqueous purslane juice on serum Cr level in CCl₄ induced hepatotoxicity rats

The liver of normal control rats showed hepatocytes, portal triads and vasculature appeared normal without histological changes (Fig. 3). The liver of treated rats with CCl₄ only revealed multifocal hepatic cell necrosis (Fig. 4). As shown in Fig. 5, liver of treated rats with CCl₄ and given orally aqueous purslane juice (5 ml/kg b. wt) has pericentral hepatic cell necrosis. However, liver of treated rats with CCl₄ and given orally aqueous purslane juice at a dose of 10 ml/kg b. wt revealed focal area of
mononuclear cells infiltration. Histological assay of the liver in treated rats with CCl₄ and given orally aqueous purslane juice at a dose of 15 ml/kg b. wt showed congested central vein in some livers (Fig. 7), while other liver have normal central veins and hepatocytes (Fig.8).

Fig. (3): Liver of normal rats showing apparently healthy hepatic histological findings (H & E X 400).

Fig. (4): Liver of positive rats showing multifocal hepatic cell necrosis (H &E histological findings (H & E X 400). X 400).

Fig. (5): Liver of treated rats with 5 ml /kg b. wt of aqueous purslane juice showing pericentral hepatic cell necrosis (H&E X 400).

Fig. (6): Liver of treated rats with 10 ml /kg b. wt of aqueous purslane juice showing focal area of mononuclear cells infiltration (H&E X 200).
Liver plays a major role in the detoxification and excretion of many endogenous and exogenous components. The impairment or damage of liver functions may affect several modulations on one's health (Ikhajiangbe et al., 2014). Once the liver is injured, its efficient treatment with renowned drugs is limited (Lee et al., 2007). Therefore, the interest of using alternative medicines for the treatment of hepatic disease has been arisen. Carbon tetrachloride (CCl4) is a highly toxic chemical substance used to induce liver damage experimentally (Karakus et al., 2011). In the present study, CCl4 was used for liver damage induction to investigate whether aqueous purslane juice as alternative medicine plant could decrease efficiently the toxicity produced in the liver.

The present results showed that serum total lipids (TL), triglycerides (TG) and total cholesterol (TC) levels were significantly increased in rats with the CCl4 treatment compare to normal control rats. These results were in accordance with Rahmat et al. (2012) and Alhassan et al. (2015) who showed that the administration of CCl4 significantly increased serum triglycerides and total cholesterol levels. These elevations indicate to the deterioration in hepatic function due to the damage caused by CCl4 administration as mentioned by (Murray et al., 1993) who reported that the extensive accumulation of lipids is observed as a pathological condition and chronic or fibrotic changes that occurs in the cells which
create cirrhosis and/or deteriorate in liver function. Boll et al. (2001) showed that the increase in serum levels of TL, TG and TC as a result of CCl4 injection is related to increase the synthesis of fatty acids and triglycerides from acetate and transport it into the liver cell, resulting in increase acetate availability. On the other hand, CCl4 lowers β-oxidation of fatty acids and hydrolysis of triglycerides. This increases the availability of fatty acids to esterification (Lieber, 2000). Moreover, the synthesis of apolipoproteins is inhibited by CCl4 and resulting in decreased the synthesis of lipoproteins (Honma and Suda, 1997). Recently, Althnaian et al. (2013) established that CCl4 treatment affecte the lipid metabolism of triglyceride and cholesterol in liver and induces a significant increase in the levels of lipid parameters. In contrast, orally administration of aqueous purslane juice at the three different doses to hepatotoxicity rats significantly reduced the elevations in serum levels of TL, TG and TC compared to that of untreated positive rats. The present results were in agreement with Park (2002) that showed ethanol extract of purslane have a lowering effect of total lipid, total cholesterol and triglyceride levels in the serum of hypercholesterolemic rats. Anusha et al. (2011) and Muneer et al. (2013) reported that the lower in serum TL, TG and TC levels are probably indicative of hepatoprotective effect of aqueous purslane juice in CCl4 administration rats. Also, several other studies indicated that the cholesterol lowering effect of purslane may be attributed to effect of it content of omega-3 fatty acid (Besong et al., 2011), higher levels of -linolenic acid and polyphenols (Oliveira et al., 2009), all of which have been shown to have a reducing effect on serum lipid levels (Kim et al., 2005). Omega-3 fatty acid is a precursor of prostaglandins hormones and may offer protection against cardiovascular disease, cancers and a number of chronic diseases and conditions throughout the human life (Mohamed et al., 2011).

With regard to serum hepatic markers, the present study showed that serum GGT, AST and ALT and ALP activities were greatly increased in the CCl4 treatment rats compare to normal control rats. These results were in agreement with Brent and Rumack (1993) who reported that the increase in serum levels of hepatic biomarkers may attributed to liver injury, because these enzymes are place in cytoplasmic area of the cell and released into circulation in the case of cellular damage. Cellular liver
damage as result of hepatotoxic effects by CCl₄ is lipid peroxidation origin and largely due to active metabolite CCl₃ causing cell injury Park et al. (2005). Several studies indicated that these enzymes activities are significantly elevated after CCl₄ treatment (Mehmetcik et al., 2008 and Arici and Cetin, 2011).

Khan and Al-Zohairy (2011) showed that CC14 increased significantly serum ALP levels. On the other hand, treatment with aqueous purslane Juice was found to suppress the increase of serum GGT, AST and ALT and ALP activities induced by CCl₄ treatment in rats. This finding implies that aqueous purslane Juice challenge to protect liver tissue from CCl₄ injury as reported by (Al-Howiriny et al., 2004) who mentioned that the protective effects of purslane extracts strongly indicated to the possibility of the purslane extracts being able to prevent and/or mitigate any leakages of marker enzymes into circulation, stimulate the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and therefore restore these enzymes levels.

Also, this study was confirmed by Mohamed et al. (2011) who reported that the decrease in serum enzymes in CCl₄-induced liver damage accordingly to aqueous purslane juice may be due to the effect in the prevention of the leakage of intracellular enzymes by its membrane stabilizing activity and have a protective role against liver damage. The hepatoprotective effects of purslane juice against CCl₄-induced oxidative stress in the rat may be related to its antioxidant properties and protect the tissues from lipid peroxidation. The antioxidant properties of purslane are related to its large amounts of phenolic compounds including coumarins, flavonoids, alkaloids, and saponins (Sakai et al., 1996). In addition, purslane was found to contain high concentrations of β-carotene, B2, C and E and is very rich in magnesium, zinc and other trace elements; which act as antioxidants and have been found to be useful in preventing toxicant-induced tissue injury (Kamal Uddin et al., 2012).

The effect of CCl₄ on developed hepatocellular damage was evident from a significant lowering in serum levels of total protein (TP) and albumin (Alb) and higher in total and direct bilirubin compared to that of the normal control rats. These results were in agreement with previous experimental studies that showed CC1₄ induced significant decrease in serum level of total protein and albumin (Fahim et al., 1999 and Khan
and Al-Zohairy, 2011). This indicate to the impair in liver functions or poor synthesis, either primary as in liver cells damage or secondary to loss protein in urine, due to nephritic syndrome and chronic glomerulonephritis induced by CCl₄ toxicity (Al-Fartosi et al., 2012). Serum bilirubin is one of the most sensitive tests employed to the diagnosis of hepatic diseases. Bilirubin is a breakdown product of hemoglobin, which transported from the spleen to the liver and excreted into bile. Hyper-bilirubinemia is related to hepatic cholestasis and hepatocellular damage Burtis, (1999).

The remarkable elevation in the rats marker bilirubin in CCl₄ administered rats in this study is agreement with Patrick-Iwuanyanwu et al., (2010). The increases in serum levels of total and direct bilirubin might be attributable to the excessive production of bilirubin as a result of excessive break down of red blood cells and the inability of animals to excrete bilirubin due to obstruction, either extra hepatic and/or intrahepatic due to damaged liver cells (Abd Elzaher, 2008). Aqueous purslane juice was showed to significantly restoration effect in the alteration of serum total protein, albumin, total and direct bilirubin levels at the entire dose levels. The present results were convenient with Walaa et al. (2011) who reported that the oral treatment of nephritic rats with purslane extract caused a very highly significant in serum total protein and albumin and reduced total and direct bilirubin concentration as compared to the nephritic control ones. The ability of purslane extract to restore the levels of TP, Alb and total and direct bilirubin might be due to its effect on the functional statues of the poisoned liver and to protect against hepatotoxicity.

In the present results, the damage in liver tissues due to CCl₄ injection was characterized by the significant increase in liver MDA concentrations and decrease in GSH, SOD and CAT activities compared to that of the normal control rats. These results were consistent with Patrick-Iwuanyanwu et al. (2007) who indicated that administration of CCl₄ caused elevate in liver MDA as a product of lipid peroxidation in liver of rats leading to tissue damage and failure of antioxidant defense mechanisms to prevent the formation of excessive free radicals. This in turn alters the ratio of polyunsaturated to other fatty acids, thus, leading to
a decrease in the membrane fluidity which may be sufficient to cause cell death.

**Muthu et al. (2008)** showed that CCl4 treatment caused a significant decrease in the level of SOD, CAT, GPx and GST in liver tissue when compared with control group. **Liu et al. (1993)** suggested that the decrease in blood and liver GSH could be the result from decreased synthesis or increased degradation of GSH by oxidative stress and tissue injury. Increased oxidative stress, resulting from a significant increase in aldehydic products of lipid peroxidation has probably decreased hepatic GSH content. Orally administration of aqueous purslane juice at the three different doses with CCl4 injection to rats significantly decreased liver concentrations of MDA and increased GSH, SOD and CAT activities towards normal rats. This result was in concordance with **O’Brien et al. (2000)** who showed elevation in liver GSH activite in the purslane extracts -treated rats. **Mohammed and Soad (2010)** indicated that the aqueous purslane extracts increase the biosynthesis of SOD, GPx, GSH and reduce the oxidative stress leading to less degradation of SOD and GPx, GSH. The present results were confirmed with **Mohamed et al. (2011)** who revealed that the hepatoprotective effect of purslane against CCl4 induced hepatotoxicity in rats may be related to the inhibition of lipid peroxidation and enhancement of antioxidant enzyme levels and free radicals scavenging action inducing significant reduction in MDA and increase in GSH, CAT and SOD activity of liver and kidney.

The effect of purslane juice in decreasing liver MDA and increasing GSH, SOD and CAT activity may be related to its antioxidant properties as reported by **Sakai et al. (1996)** who mentioned that the mechanisms exerted by purslane in inhibits liver damage may be due to its large amounts of phenolic compounds, including coumarins, flavonoids, alkaloids, and saponins that contribute towards the antioxidant activity. **Rathee et al. (2006)** indicated that phytochemical compounds of purslane are capable of modulating the activity of SOD and GPx enzymes and affecting the action of many cell systems and possess a significant anti-hepatotoxic, and anti-oxidant activities. On the other hand, **Simopoulos et al. (2009)** reported that the inhibition effect of purslane in liver damage may be due to its contain omega-3 fatty acids.
The effect of CCl4 injection on the kidney function biomarkers showed significant elevation in BUN and Cr levels in the CCl4 treated group compared to that of the normal control rats. The present result was in agreement with Muhammad et al. (2009) who found that renal disorders in rat indicated by the presence of abnormally high levels of BUN in serum, urobilinogen in urine and creatinine both in urine and serum which are possible indicators of hepatic and/or kidney injuries induced through CCl4 treatment. However, values of BUN and Cr were significantly corrected towards the control values in rats intoxicated with CCl4 and treated with aqueous purslane juice at the three different doses.

Nitha and Janardhanan (2008) and Walaa et al. (2011) showed that the treatment of gentamicin intoxicated rats with purslane extract made decreasing in serum creatinine and serum urea level. Decreasing levels of urea, uric acid and creatinine in the purslane treated animals, may be due to its antioxidant potential (Shirwaikar et al., 2003).

The biochemical findings were confirmed by histological observations. The changes in untreated hepatotoxicity rats (positive group) mostly include hepatocellular necrosis which was in coincidence with the findings of (Sun et al. (2001) and Althnaian et al. (2013). While, treated with purslane juice tends to improve liver tissues, which more careful with higher level. The present results have clearly demonstrated the ability of purslane juice to decrease oxidative stress in rat liver, as evidenced by the very highly significant decrease of lipid peroxidation product; and a very highly significant rise of endogenous antioxidants GSH, SOD and CAT these findings are in agreement with other reports (Shirwaikar et al., 2003 and Rathee et al., 2006).

Conclusion:

In conclusion, the results of this study demonstrated that aqueous purslane juice possess a potent hepatoprotective action upon CCl4-induced hepatic damage in rats. This may be due to its antioxidative activity with its ability to scavenge free radicals and inhibit lipid peroxidation, all of which are capable of hepatocellular injury. These results affirm that the traditional use of purslane plant for the protection of hepatotoxicity may be beneficial for patients and hence it is worth studying on humans.
References:


المجلة الدراسات وبحوث التربية النوعية

التأثيرات الواقية لعصير الرجلة المائي على ذكور الفئران المصابة

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الملخص:

نبات الرجلة من الفصيلة الرجلية الذي يستخدم على نطاق واسع في الطب الشعبي كمضاد لمرض السكر وانخفاض ضغط الدم. وقد تم تصميم هذه التجربة لدراسة التأثير الواقيق لعصير الرجلة المائي على ذكور الكركدون. وقد استخدم في هذه التجربة عدد خمسة وثلاثين من ذكور الفئران والتي تم تقسيمها إلى خمسة مجموعات وتحتوي كل مجموعة على خمسة فئران. واستخدمت المجموعة الأولى كنوعية ضابطة طبيعية، بينما المجموعات الثانية، الثالثة، الرابعة والخامسة تم حقنها تحت الجلد برابع كلوريد الكركدون (20 ملليغرام/كم من وزن الفئران) وذلك لإحداث التسمم الكبدى. واستخدمت المجموعة الثانية كمجموعة ضابطة إيجابية، بينما تم معالجة المجموعات الثالثة، الرابعة والخامسة عن طريق إعطاءها جرعات مختلفة من عصير الرجلة (5، 10 و15 مل/كم من وزن الجسم- علي التوالي) عن طريق الفم. وتضمن تكريرات صورة دهون الدم، وتوزن الكلي، كما تم تغيير تركيز المكلدانا الدهي وتركيز أنشطة إنزيمات الجلودتان المختل، السوبر أوكسيد ديسبيوزيتز، الكتاليز في الكبد. تم اجراء الفحص الهستوبولوجي لأنسجة الكركدون لتحديد مدى الضرر الناجم عند استخدام رابع كلوريد الكركدون. وأظهرت النتائج وجود زيادة كبيرة واضحة في تركيزات السيرم من الدهون الكلية، الدهون الثلاثية، الكولسترول، البلاكوبين الكلي والمباشر، البوريا نيتروجين ونترترات، إنزيمات الكبد من الجاما جلوتوميل ترانسفيراز، الأسترات ترانس أميين، الأثنين أمينو ترانسفيراز، الإنزيمات الفوسفاتاز. كذلك وجود زيادة كبيرة في محتوى الكركد من المانو داي الدهيد. بينما وجد نقص واضح في تركيزات السيرم من البروتينات الكلية والألبومين. ووجود نقص واضح في محتوي الكركد من إنزيمات الجلودتان المختل، السوبر أوكسيد ديسبيوزيتز، الكتاليز. وأظهرت نتائج أن معالجة الفئران المصابة بتسمم الكركد بعصير الرجلة المائي يؤدي إلى تحسين مستوي المؤثرات البيوكيميائية السابقة حيث يزداد التحسين

المجلة الثاني- العدد الأول- مسلسل العدد (3) - يناير 2012 - 277
زيادة الجرعة المأخوذة من العصير. كما أظهرت نتائج الفحص الفيسيولوجي للأنسجة الكبدية وجود العديد من الخلايا الكبدية الميتة، بعض الخلايا الميتة في البؤر الكبدية. ارشاح في بعض المناطق بالكبد وذلك في الفئران المصابة بتسمم الكبد والغير معالجة. الفئران المعالجة بـ 10 مل/كم م من عصير الرجلة المائي علي التوالي. كما أظهرت نتائج الفحص للكبد الفئران المصابة والمعالجة بجرعة 15 مل/كم م من عصير الرجلة وجود احتقان في الوريد المركزي في بعض العينات من الكبد وكذلك ظهور خلايا كبدية طبيعية في البعض الآخر. في النهاية أكدت الدراسة على أن عصير الرجلة المائي له تأثير مضاد لتمد الكبد في الفئران. هذه النتائج تؤكد على أن الاستخدام التقليدي للنباتات ال الرجلة هام للوقاية من تسوس الكبد والتي قد تكون مفيدة لمرضى الكبد، وبالتالي فإن الأمر يستحق الدراسة على البشر.

الكلمات الاسترشادية: رابع كيوريك الكربون، تسمم الكبد، وظائف الكبد والكلي، الإنزيمات المضادة للأكسدة.