American Journal of Pharmacology and Toxicology 5 (4): 167-176, 2010
ISSN 1557-4962

Evaluation of *in vivo* Antioxidant and Hepatoprotective Activity of *Portulaca oleracea* L. against Paracetamol-Induced Liver Toxicity in Male Rats

Mohamed H. Ahmida
Department of Nutrition, Public Health Faculty, Garyounis University, Benghazi-Libya

Abstract: Problem statement: Oxidative stress plays a major role in paracetamol induced hepatotoxicity. *Portulaca oleracea* (P.O.) has been shown to exhibit antioxidant properties. The present study aimed to investigate the effect of P.O. on the oxidative stress in Paracetamol-induced hepatic toxicity in male rats. **Approach:** Forty male rats were divided into four groups. Group I served as control; Group II Received isotonic saline for 20 days and then simultaneously administered with paracetamol 750 mg kg^{-1} body wt. every 72 h for 10 days; Group III received freshly prepared P.O. for 30 days; Group IV received freshly prepared P.O. for 20 days and then simultaneously administered with paracetamol 750 mg kg^{-1} body wt. every 72 h for 10 days. All animals were sacrificed cervical decapitation 24 h after the last application and the blood was collected for the determination of serum marker of lipid peroxidation, antioxidant and histology of the liver was performed. **Results:** Paracetamol treatment resulted in an increase in the hepatic TBARS content and depletion in total antioxidant capacity, reduced glutathione content, catalase and superoxide dismutase activities. Administration of P.O. with paracetamol significantly ameliorated the indices of hepatotoxicity induced by paracetamol. In addition, P.O. alleviated paracetamol induced oxidative changes in liver. **Conclusion:** The present study demonstrated that P.O. inhibits paracetamol-induced hepatotoxicity and might serve as a protective agent with paracetamol to limit its free radical induced liver injury.

Key words: Oxidative stress, *Portulaca oleracea*, hepatoprotection, Superoxide Dismutase (SOD), Reactive Oxygen (ROS), N-Acetyl-P-benzoquinone Imine (NAPQI), Serum Aspartate Transaminase (AST), Alanine Transaminase (ALT)

INTRODUCTION

Paracetamol is a commonly used analgesic and antipyretic drug. The drug is safe at therapeutic levels, but an acute paracetamol overdose can lead to potentially fatal hepatic necrosis in humans and experimental animals (Proudfoot and Wright, 1970; Thomas, 1993). It is becoming clear that Reactive Oxygen (ROS) and Nitrogen (RNS) species take an important part in the development of hepatotoxicity caused by paracetamol (Nakae *et al*., 1990; Michael *et al*., 1999; Knight *et al*., 2001). The initial step of its toxicity is cytochrome P450 metabolism of paracetamol to the reactive intermediate N-Acetyl-P-benzoquinone Imine (NAPQI) (Dahlin *et al*., 1984). At therapeutic doses this metabolite is removed by conjugation with Glutathione (GSH). However at large doses of paracetamol, conjugation with GSH leads to its depletion (Mitchell *et al*., 1973a). This paracetamol-induced hepatic toxicity was prevented by antioxidants and also cytochrome P-450 inhibitors (Sener *et al*., 2003; Abraham, 2005). It is logical to consider antioxidants as primary candidates to counteract such toxic effect. An accumulating evidence supported the protective effects of antioxidants from medicinal plants against oxidative stress–mediated disorders. Studies are going on throughout the world for the search of protective molecules that would provide maximum protection of the liver, kidney as well as other organs and practically very little or no side effects would be exerted during their function in the body (Montilla *et al*., 2005; Mansour *et al*., 2006).

Portulaca oleracea L. (Purslane) (Portulacaceae) (P.O.) is a grassy plant with small yellow flowers and stems sometimes flushed red or purple, which grows widely in different areas of the world including Libya (Jin *et al*., 2007). The plant contains many biologically active compounds, including free oxalic acids, β-Carotene, omega-3 fatty acids, coumarins, flavonoids, monoterpene glycoside and

anthraquinone glycosides (Hai-Liang *et al*., 2008; Boroushaki *et al*., 2004). P.O. has a long history of use for human food, animal feed and medicinal purposes. The whole plant is edible, either raw or cooked. It is used in liver and kidney disorders, as an emollient, astringent and as a diuretic in Arab traditional medicine (Ghazanfar, 1994). P.O. also used for alleviating pain and swelling (Okwuasaba *et al*., 1987). The entire plant is usually cut into small pieces and eaten with salt; applied topically to soothe skin (Ghazanfar, 1994). It also exhibits a wide range of pharmacological effects, including antibacterial (Zhang *et al*., 2002), analgesic, anti-inflammatory and skeletal muscle- relaxant activities (Parry *et al*., 1987; Parry *et al*., 1993). A bronchodilatory effect in asthmatic patients, skeletal muscle relaxant and antifertility effect were also reported for P.O. (Elkhayat *et al*., 2008). It has been described as a "power food" of future because of its high nutritive and antioxidant properties (Simopoulos *et al*., 1995). The hepatoprotective activities of hydroalcoholic extract of stems and leaves of P.O. in rats treated with CCl_4 (Elkhayat *et al*., 2008) or rifampicin (Kulkarni *et al*., 2007) were previously reported. The present study was designed to evaluate the protective effects of Portulaca oleracea against Paracetamol-induced hepatic toxicity in male rats. Liver toxicity was induced by administrating Paracetamol in the presence or absence of P.O.

This study includes biomarkers of hepatic toxicity such as histopathological study and serum activity of AST, ALT, ALP and γ-GT. Oxidative stress and antioxidants of liver will study by determination of reduced glutathione (GSH), Superoxide Dismutase (SOD) and catalase (CAT) enzyme activities and lipid peroxidation (Thiobarbituric acid reactive substances, TBARS).

MATERIAL AND METHODS

Chemicals: Paracetamol was supplied by Control Center for Food and Medicines, Benghazi, Libya. Commercial kits to estimate serum hepatic markers were from Randox laboratories Ltd. (Admore, Crumlin, Co-Antrim, UK). All other chemicals and biochemicals were of analytical grade obtained from local firms.

Plant material: The aerial parts of freshly obtained P.O. from local vegetable market of Benghazi were subjected to obtain juice using an electrical blender. The fresh juice was then used in a dose of 300 mg kg^{-1} body weight in all experiments.

Animals: Forty healthy adult male Wistar albino rats (The central animal house of Garyounis University, Benghazi, Libya) weighting between 200 and 250 g were used. We used only male rats because of their constant metabolism compared to the variation in the female physiology. The animals were housed under standard laboratory conditions (12 h light and 12 h dark) in a room with controlled temperature (24 ± 3°C) and humidity (55%) during the experimental period. All experimental procedures involving animals were conducted in accordance with the guidelines of National Institutes of Health (NIH guidelines).The study protocol were approved by Ethical committee of Garyounis University. The rats were provided ad libitum with tap water and fed with standard commercial rat chow (pellet form, in the sack, Benghazi Animal Feed Company, Benghazi, Libya). The rats were acclimatized to laboratory condition for 7 days before commencement of experiment. The animals were randomly divided into four groups containing teen rats each one. Paracetamol was orally administred to animals with dose 750 mg kg^{-1} (Araya *et al*., 1987) at every 72 h for 10 days. P.O. was freshly prepared and administered to animals by oral gavages with a dose of 300 mg kg^{-1} day^{-1}. The dose of P.O. used in this study was selected on the basis of the previous study.

Experimental design:

Group 1 (Control) received an orally isotonic saline for 30 days;
Group 2 Received isotonic saline for 20 days and then simultaneously administered with paracetamol 750 mg kg^{1} body wt. every 72 h for 10 days;
Group 3 Received freshly prepared P.O. for 30 days;
Group 4 Received freshly prepared P.O. for 20 days and then simultaneously administered with paracetamol 750 mg kg^{-1} body wt. every 72 h for 10 days.

Sample collection and biochemical assays: The animals in all groups were sacrificed by cervical decapitation 24 h after the last application. Blood samples were collected into the tubes without adding anticoagulant. The samples were centrifuged at 200×g for 5min at +4°C to separate their serum. The serum was stored at -80°C after separation until it was assayed as described below.

Assessment of serum marker enzymes: The activities of Serum Aspartate Transaminase (AST) and Alanine Transaminase (ALT) were determined using the method of Reitman and Frankel. Serum alkaline phosphatase (ALP) was determined according to the method of Klein *et al*. (1960), Lactate Dehydrogenase (LDH) was assayed spectrophotometrically according to the method of Bergmeyer *et al*. (1974). γ-Glutamyl transferase (GGT) activity was determined by the method of using γ-glutamyl-p-nitroanilide as substrate.

Protein determination: Serum protein was determined by the method of Lowry *et al*. (1951) using bovine serum albumin as a standard.

Assessment of antioxidant enzymes: The liver was dissected out; weighed and washed using cooled saline solution. The homogenization of tissues was carried out in Teflon-glass homogenizer with a 5-10 mL buffer containing 50 mM potassium phosphate, pH 7.5 and 1mM EDTA per gram tissue. Homogenates were centrifuged at 10,000×g (+4°C) for 15 min. The resulting clear supernatant was used for various enzymatic assays as described below.

Catalase (CAT) activity was assayed colorimetrically as described by Sinha (1972) using dichromate-acetic acid reagent and was expressed as U (μmol of H_2O_2 utilized/min) /mg protein. Superoxide dismutase assay (SOD) activity was assayed according to Beyer and Fridovich (1987). The assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. SOD activity was expressed in Unit enzyme/mg protein. One unit of SOD is defined as 1μg of SOD needed for half-maximum inhibition of NBT in the assay.

Assessment of lipid peroxidation enzymes: Induction of lipid peroxidation by paracetamol and its protection by the P.O. was determined by the estimation of various enzyme activities and TBARS contents.

Glutathione S-transferase (GST) activity was determined spectrophotometrically by using dichloro-2,4-dinitrobenzene as the substrate (Habig *et al*., 1974) and was expressed as U (μmol of CDNB-GSH conjugate formed/min)/mg protein. Glutathione Reductase (GR) activity was measured at 340 nm by spectrophotometry and the amount of the enzyme reducing 1 μmol GSSG per min was regarded 1 activity unit as Carlberg and Mannervik described (Carlberg and Mannervik, 1985). Glutathione Peroxidase (GPx) activity was determined by the procedure described by Paglia and Valentine (1967). The procedure of analysis performed was based on the oxidation of reduced GSH by GPx coupled to the disappearance of NADPH by GSH-reductase measured at 37°C and 340 nm and was expressed as U (nmol of NADPH oxidized min^{1}) mg^{1} protein. Tissue GSH concentration was measured by a kinetic assay using a dithionitrobenzoic acid recycling method described by Ellman (1959) and was expressed as μmol g^{-1} tissue. The concentrations of TBARS were determined according to a modified method of Ohkawa *et al*. (1979) based on the reaction with thiobarbituric acid and were expressed as nmol g^{-1} tissue.

Histological assessment: Livers from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with Hematoxylin Eosin (H&E) for light microscopic analyses. The slides were coded and were examined by a histopathologist who was ignorant about the treatment groups after which photographs were taken.

Statistical analysis: The results are presented as mean ± S.E. Analyses were performed using students t-test and Duncan's multiple range tests for the difference between the control and treatment groups. $P<0.05$ was considered statistically significant.

RESULTS

Serum hepatic marker enzymes status: The effects of paracetamol on the Serum enzymes AST, ALT, ALP, LDH and γ-GT are shown in Table 1. Oral administration of paracetamol significantly increased the activities of the serum enzymes by 123.03%, 225.70, 96.77, 176.07 and 334.70%, respectively, when compared with the control ($p<0.05$). Pre and simultaneous treatment of the rats with P.O. (300 mg kg^{1}) ameliorated these increases significantly by 49.46, 63.94, 44.73, 62.35 and 57.84% in AST, ALT, ALP, LDH and γ-GT respectively, when compared with the paracetamol-treated group. Restoration of hepatic marker enzymes was at maximum in the higher dose level (300 mg kg^{-1}) of P.O. than the lower dose level (150 mg kg^{-1}) when compared with paracetamol-treated rats. Based on these findings 300 mg kg^{-1} of P.O. was fixed as a dose for further tissue biochemical investigations.

Table 1: The influence of oral administration of *Portulaca oleracea* (P.O.) (300 mg kg^{-1} day^{-1}) on serum AST, ALT, ALP, LDH and γGT in paracetamol-treated rats

Treatment	AST (U/L)	ALT (U/L)	ALP (U/L)	LDH (U/L)	γGT (U/L)
Control	63.87±3.23	47.05±2.61	175.08±6.30	45.84±2.83	2.45±0.46
P.O. (300 mg kg^{-1})	67.89±3.61	46.56±3.09	180.76±5.34	46.89±2.81	3.00±0.88
Paracetamol (750 mg kg^{-1})	142.45±4.35*	153.24±4.21*	344.50±13.44*	126.55±5.13*	10.65±1.91*
P.O. (300 mg kg^{-1})+ (750 mg kg^{-1}) Paracetamol	72.00±2.91**	55.26±2.89**	190.39±5.90**	47.65±2.43**	4.49±0.92**

Data are expressed as mean ± S.E.; *: Significance difference from control group at p<0.05; **: Significance difference from paracetamol-intoxicated group at p<0.05; Number of rats per group n = 10

Table 2: The influence of oral administration of *Portulaca oleracea* (P.O.) (300 mg kg^{-1} day^{-1}) to paracetamol-induced lipid peroxidation and reduced glutathione (GSH) content in the liver of rats

Treatment	TBARS	GSH
Control	44.00±1.38	3.95±0.22
P.O. (300 mg kg^{-1})	43.00±1.61	3.90±0.12
Paracetamol (750 mg kg^{-1})	82.00±3.60*	1.20±0.24*
P.O. (300 mg kg^{-1})+(750 mg kg^{-1}) Paracetamol	49.00±1.09**	3.59±0.17**

Data are expressed as mean ± S.E.; *: Significance difference from control group at p<0.05; **: Significance difference from paracetamol-intoxicated group at p<0.05; Number of rats per group n = 10; TBARS = Thiobarbituric Acid Reactive Substance

Table 3: The influence of oral administration of *Portulaca oleracea* (P.O.) (300 mg kg^{-1} day^{-1}) to paracetamol-treated rats on antioxidant enzyme activities [Superoxide Dismutase, (SOD); Catalase, (CAT); Glutathione-S-Transferase, (GST); Glutathione Peroxidase, (GPx) and Glutathione Reductase (GR)] of paracetamol-intoxicated rat liver homogenate

Treatment	SOD	CAT	GST	GPx	GR
Control	7.48±0.32	56.50±3.00	2.45±0.11	5.23±0.38	0.28±0.02
P.O. (300 mg/kg)	7.90±0.36	59.00±4.10	2.69±0.15	5.14±0.40	0.29±0.02
Paracetamol (750 mg kg^{-1})	5.10±0.43*	35.50±2.91*	1.45±0.06*	2.56±0.21*	0.16±0.03*
P.O. (300 mg kg^{-1}) + (750 mg kg^{-1}) Paracetamol	6.94±0.31**	54.50±3.33**	2.41±0.11**	4.81±0.42**	0.24±0.02**

Data are expressed as mean ± S.E.; *: Significance difference from control group at p<0.05; **: Significance difference from paracetamol-intoxicated group at p<0.05; Number of rats per group n = 10; SOD: one unit of activity was taken as the enzyme reaction,which gave 50% inhibition of NBT reduction in 1min/mg protein; CAT: µmol of H2O2 utilized/min mg protein; GST: µmol of CDNB-GSH conjugate formed/min/mg protein; GPx: µg of GSH consumed/min mg protein; GR: nmol of NADPH oxidized/min mg protein

Lipid peroxidation and hepatic Glutathione content status: The changes in the levels of hepatic lipid peroxidation and reduced glutathione (GSH) content in control and experimental rats are shown in Table 2. The levels of Thiobarbituric Acid Reactive Substances (TBARS) were significantly increased (p<0.05) in paracetamol-treated rats when compared with normal control rats. Pre and simultaneous oral administration of P.O. (300 mg kg^{-1}) along with paracetamol significantly lowered the levels of TBARS in the liver of rats when compared to paracetamol-treated rats. A significant depletion (p<0.05) in the level of GSH was noticed in rats treated with paracetamol when compared to normal control rats. Treatments with P.O. (300 mg kg^{-1}) significantly (p<0.05) restored the level of GSH to near normalcy when compared with paracetamol-treated rats.

Hepatic antioxidant enzymes status: Table 3 illustrates the levels of enzymatic antioxidants namely Superoxide Dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), Glutathione-S-Transferase (GST) and the glutathione metabolizing enzyme (Glutathione Reductase) (GR) in the liver of control and experimental rats. A significant decrease (p<0.05) in the activities of hepatic–enzymatic antioxidants in paracetamol-treated rats was seen. Treatment with P.O. (300 mg kg^{-1}) along with paracetamol significantly increased the activities of enzymatic antioxidants in liver when compared with paracetamol-treated rats.

Histopathology of the liver: Histopathological examination of the liver sections from normal rats showed normal parenchymal architecture; no significant lesions were observed (Fig. 1A). In the rats treated with paracetamol alone, cloudy swelling, fatty degeneration, hepatocellular necrosis, heavy haemorrhage and irregular appearance due to cell death were seen (Fig. 1B). The above changes were reduced in the liver of rats treated with P.O. and paracetamol together (Fig. 1C). The histological pattern were almost normal in rats treated with P.O. alone (Fig. 1D).

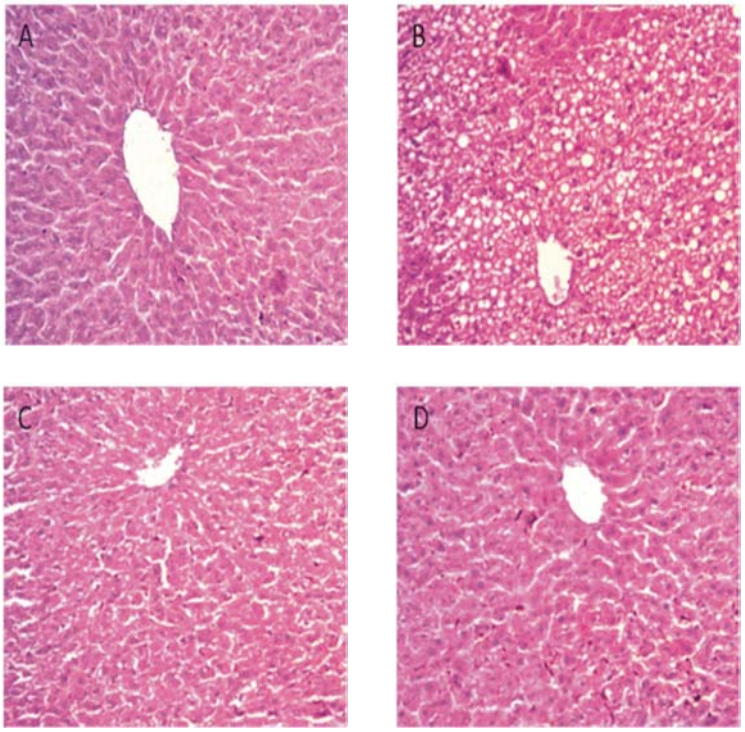

Fig. 1: Representative photographs from the liver showing the protective effect of *Portulaca oleracea* (P.O.) on paracetamol-induced hepatic injury in rats. (A). Control rat liver. Normal hepatic parenchyma. (B) Paracetamol-treated rat liver showing cloudy swelling, fatty degeneration of hepatocytes, with necrosis, heavy haemorrhage, irregular appearance and damaged central vein (C) P.O. (300 mg kg^{-1} day^{-1}) + paracetamol-treated rat liver. Normal appearance of hepatocytes with mild sinusoidal dilation. (D) P.O. (300 mg $kg^{-1}day^{-1}$) treated rat liver showing near-normal appearance of hepatocyte saround the central vein

DISCUSSION

Hepatotoxicity is the potential complication of paracetamol, which is widely, used in general medicine and an assessment of its relative toxicity is important. The primary toxicity of paracetamol is the result of drug metabolism in liver (Gu *et al*., 2005). At therapeutic doses, paracetamol is metabolized via glucuronidation and sulfation reactions result in the water-soluble metabolites that are excreted via the kidney. The result of the metabolic conversion of paracetamol by the microsomal P-450 enzyme system is that, a highly reactive intermediate, namely, N-Acetyl-P-Benzoquinone Imine (NAPQI) is produced. This metabolite is then reduced by Glutathione (GSH) (Bessems and Vermeulen, 2001). When large doses of paracetamol are ingested, there is more severe GSH depletion as well as massive production of metabolites, which compounds the toxicity, leaving large amounts of reactive metabolite unbound. These intermediates then form covalent bindings with macromolecules on cellular protein (Bessems and Vermeulen, 2001). This process disrupts homeostasis and initiates apoptosis, or programmed cell death, leading to tissue necrosis and ultimately to organ dysfunction. Depletion of hepatic GSH is one of the primary factors which permit lipid peroxidation, suggested to be closely related to paracetamol liver damage. Therefore protection against the paracetamol toxicity can be achieved through the supplementation of antioxidants (Sener *et al*., 2003). In this context the present study also confirmed that the administration of P.O. (300 mg kg^{-1}) significantly preserved the hepatic function against the hepatotoxic effect exerted by paracetamol over dose. Liver injury followed by paracetamol administration is well established by the elevated levels of serum hepatic marker enzymes indicating the cellular leakage and loss of functional integrity of hepatic membrane architecture. High levels of AST and ALT are the crucial parameters to detect liver damage (Benjamin, 1978; Benjamin, 1984). It correlates with the present study which revealed that the increased activities of

AST and ALT in the serum of paracetamol treated rats. Serum ALP levels are also related to the status and function of hepatic cells. Increase in serum alkaline phosphatase is due to increased synthesis in the presence of increasing biliary pressure (Moss and Butterworth, 1974). Changes in the activities of these enzymes are liver specific and have been considered as a tool to study varying cell viability and cell membrane permeability (Yemitan and Izegbu, 2006). Serum LDH, a cytoplasmic marker enzyme and γGT, a membrane bound enzyme are the well-known indicators of cell and tissue damage by toxic substances and their levels are also substantially increased in paracetamol- intoxicated rats. LDH is an intracellular enzyme, the increase of which in serum is an indicator of cell damage (Navarro and Senior, 2006). In the present study increased levels of serum hepatic markers suggested that an extensive liver injury was occasioned by paracetamol due to increased lipid peroxidation which have the ability to cause membrane damage. Paracetamol causes structural and functional damage to the cell membrane and increased the membrane permeability leading to the leakage of hepatic enzymes into the blood. It is well established that paracetamol toxicity significantly elevated the serum hepatic marker enzymes (Maryann and Jeanmarie, 2008; Girish *et al*., 2009). Administration of P.O. (300 mg kg^{-1}) attenuated paracetamol-induced hepatotoxicity as shown by the decreased levels of AST, ALT, ALP, LDH and γGT thus offering protection against paracetamol hepatotoxicity in rats. The above effects clearly indicate that P.O. may offer protection by stabilizing the cell membrane in hepatic damage induced by paracetamol. Furthermore it has been reported that P. O. decreases the liver marker enzymes during carbone tetrachloride-induced hepatotoxicity.

Paracetamol is known to produce oxidative damage in the liver by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals (James *et al*., 2003). The free radicals attack the cell membrane, thus leading to destabilization and disintegration of the cell membrane as a result of lipid peroxidation (Shakun and Vysotski, 1982). In the present study,we observed a marked elevation of hepatic TBARS content following paracetamol administration was in consistence with the other reports in paracetamol- intoxicated rats (James *et al*., 2003; Botta *et al*., 2006; Sener *et al*., 2006). Constituents of P.O. such as flavonoids (quercetin), polyphenolic alkaloids (Oleracin A, B and E), omega-3, ascorbic acid, β- carotene and glutathione scavenge a wide range of free radicals including the most active hydroxyl radical, which may initiate lipid peroxidation. It prevents the loss of lipophilic antioxidant α-tocopherol, by repairing tocopheryl radicals and protection of the hydrophilic antioxidant ascorbate (Yang *et al*., 2009) and increasing intracellular concentration of glutathione. Therefore, it may decrease the concentration of lipid free radicals and terminate initiation and propagation of lipid peroxidation (Zeng, 1999).

Hepatic peroxides are reported to be increased during oxidative stress (James *et al*., 2003). Seven *et al*. (2004) also, reported that, due to liver damage, there was an observed decrease in antioxidant defenses in the liver. The impaired regeneration of protective and antioxidants such as reduced glutathione also contribute to oxidative stress (James *et al*., 2003). GSH is a sulfhydryl peptide enormously present in all biological systems. It forms the first line of defense against oxidative insult by acting as a non-enzymatic antioxidant by direct inter- action of its sulfhydryl group with ROS or it can be involved in the enzymatic detoxification reaction of ROS as a cofactor or as a coenzyme. Metabolic conversion of paracetamol to NAPQI which reduced by glutathione leading to its inactivation (James *et al*., 2003). In the present study, the depressed levels of GSH in paracetamol toxicity might increase the susceptibility of the liver to free radical damage. Our findings are in consonance with the other published reports which quoted that GSH concentration is decreased during paracetamol intoxication (Mitchell *et al*., 1973b; Muriel *et al*., 1992).

The antioxidant enzymes, CAT is a major antioxidant enzyme with hematin as the prosthetic group and it is ubiquitously present in all aerobic cells containing a cytochrome system. It is most abundant in the liver and is responsible for the catalytic decomposition of H_2O_2 to oxygen and water. SOD is an extremely effective antioxidant enzyme and is responsible for catalytic dismutation of highly reactive and potentially toxic superoxide radicals to H_2O_2 (Sarsilmaz *et al*., 2003). GPx is a selenoenzyme, which plays a major role in the reduction of H_2O_2 and hydroperoxide to non-toxic products (Freeman and Crapo, 1982).

Paracetamol adminstration decreased the activities of these enzymes and GSH concentration in the tissues (Cekmen *et al*., 2009). Administration of P. O. increased the activities of SOD, CAT and GPx in the liver of paracetamol-treated rats, which might be due to the ability of P. O. to reduce the accumulation of free radicals generated during paracetamol-induced lipid peroxidation. Zeng (1999) demonstrated that P. O. protects cells from reactive oxygen species-mediated

cell death via its antioxidant activity. The antioxidant activity or the inhibition in the generation of free radical is important in providing protection against hepatic damage (Shahjahan *et al*., 2004).

Glutathione Reductase (GR) is the enzyme responsible for the reduction of oxidized glutathione (GSSG) to GSH. Glutathione S-Transferase (GSTs) are a group of multifunctional isoenzymes located both in the cytosol and the endoplasmic reticulum of the liver and plays an important role in the detoxification of toxic electrophiles by catalyzing the conjugation of these electrophiles with glutathione to form more water soluble compounds (Boyer *et al*., 1984). GST is considered as first line of defense against oxidative injury along with other antioxidant enzymes, decomposing O_2 and H_2O_2 before interacting to form the more reactive hydroxyl radicals (Chasseaud, 1979).

In the present investigation the level of reduced glutathione and the activities of glutathione-dependent enzymes (GR and GST) were reduced significantly in rats administered with paracetamol. The depression in GSH contents along with GST activity makes the cells more susceptible to by toxic electrophilic compounds. The overproduction of ROS as indicated by the elevated level of TBRAS by paracetamol in the present study may be associated with the depletion of GSH level and GST activity. Administration of P.O. along with paracetamol significantly elevated the levels of glutathione and glutathione-metabolizing enzymes, P.O. contributed significantly to the intracellular antioxidant defense system by acting as a powerful consumer of singlet oxygen and hydroxyl radicals.

In the present study, the hepatic histoarchitecture of the paracetamol-treated rats resulted severe necrotic changes, inflammatory cell infiltration, fatty degeneration and vacuolization. It might be due to the formation of highly reactive radicals and subsequent lipid peroxidation induced by paracetamol. The accumulated hydroperoxides can cause cytotoxicity, which is associated with the peroxidation of membrane phospholipids by lipid hydroperoxides, the basis for hepatocellular damage. The necrotic conditions coincide with our biochemical observations, which showed the increased level of lipid peroxidation. Administration of P.O. reduced the histological alterations provoked by paracetamol quite appreciable. It can be attributed to the antiradical/ antioxidant efficacy of P.O. which significantly reduced the oxidative stress leading to the reduction of histopathological alterations and restoration of normal physiological state of the liver.

CONCLUSION

In conclusion, the results of this study demonstrated that P.O. protect against the paracetamol-induced liver damage in rats. These results show that the protective effect of the P.O. may be to its antioxidant activities and due to their ability to decrease the metabolic activation of paracetamol. The present results could lead to a possible development of a therapeutic remedy to limit or at least alleviate some of the reported paracetamol-induced side effects in human.

ACKNOWLEDGEMENT

Thanks to Miss A. Alya for her involvement in histopathological examination and Mr A. Abusree for animal care assistance.

REFERENCES

Abraham, P., 2005. Vitamin C may be beneficial in the prevention of paracetamol-induced renal damage. Clin. Exp. Nephrol., 9: 24-30. DOI: 10.1007/s10157-004-0335-6

Araya, H., T. Horie, M. Hayashi and S. Awazu, 1987. An alteration in the liver microsomal membrane of the rat following paracetamol overdose. J. Pharm. Pharmacol., 39: 1047-1049. PMID: 2894438

Benjamin, M.M., 1984. Manuel de patologia clinica veterinaria. 1st Edn., Limusa, USA., ISBN-10: 9681816927, pp: 421.

Benjamin, M.M., 1978. Outline of veterinary clinical pathology. 3rd Edn., Iowa State University Press, USA., ISBN-10: 0813812305, pp: 351.

Bergmeyer, H.U., K. Gawehn and P Lund, 1974. 2nd Edn., Methods of Enzymatic Analysis. Verlag Chemie, USA., 3527255966, pp: 2302.

Bessems, J.G.M. and N.P.E. Vermeulen, 2001. Paracetamol (acetaminophen)-induced toxicity: molecular and biochemical mechanisms, analogues and protective approaches. Crit. Rev. Toxicol., 31: 55-138. DOI: 10.1080/20014091111677

Beyer, Jr., W.F. and I. Fridovich., 1987. Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal. Biochem., 161: 559-566. DOI: 10.1016/0003-2697(87)90489-1

Boroushaki, M.T., M.H. Boskabadyb and F. Malek, 2004. Antitussive effect of *Portulaca oleracea* L. in Guinea Pigs. Iran. J. Pharmaceut. Res., 3: 187-190.

Botta, D., S. Shi, C.C. White, M.J. Dabrowski and C.L. Keener *et al*., 2006. Acetaminophen-induced liver injury is attenuated in male glutamate-cysteine ligase transgenic mice. J. Biol. Chem., 281: 28865-28875. DOI: 10.1074/jbc.M605143200

Boyer, T.D., D.A. Vessey, C. Holcomb and N. Saley, 1984. Studies of the relationship between the catalytic activity and binding of non-substrate ligands by the glutathione S-transferases. Biochem. J., 217: 179-185. PMCID: PMC1153195

Carlberg, I. and B. Mannervik, 1985. Glutathione reductase assay. Methods Enzymol., 113: 484- 495.

Cekmen, M., Y.O. Ilbey, E. Ozbek, A. Simsek and A. Somay *et al*., 2009. Curcumin prevents oxidative renal damage induced by acetaminophen in rats. Food Chem. Toxicol., 47: 1480-1484. DOI: 10.1016/j.fct.2009.03.034

Chasseaud, L.F., 1979. The role of glutathione and glutathione S-transferases in the metabolism of chemical carcinogens and other electrophilic agents. Adv. Cancer Res., 29: 175-274. DOI: 10.1016/S0065-230X(08)60848-9

Dahlin, D.C., G.T. Miwa, A.Y. Lu and S.D. Nelson, 1984. N-acetyl-p-benzoquinone imine: A cytochrome P-450-mediated oxidation product of acetaminophen. Proc. Natl. Acad. Sci. USA., 81: 1327- 1331. PMID: 6424115

Elkhayat, E.S., S.R.M. Ibrahim and M.A. Aziz, 2008. Portulene, a new diterpene from Portulaca oleracea L. J. Asian Nat. Prod. Res., 10: 1039-1043. DOI: 10.1080/10286020802320590

Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys., 82: 70-77. DOI: 10.1016/0003-9861(59)90090-6

Freeman, B.A. and J.D. Crapo, 1982. Biology of disease: Free radicals and tissue injury. Lab. Invest., 47: 412-426. PMID: 6290784

Ghazanfar, S.A., 1994. Handbook of Arabian Medicinal Plants. 1st Edn., CRC Press, China, ISBN-10: 084930539X, pp: 265.

Girish, C., B.C. Koner, S. Jayanthi, K.R. Rao, B. Rajesh *et al*., 2009. Hepatoprotective activity of six polyherbal formulations in paracetamol induced liver toxicity in mice. Indian J. Med. Res., 129: 569-578. PMID: 19675387

Gu, J., H. Cui, M. Behr, L. Zhang and Q.Y. Zhang *et al*., 2005. *In vivo* mechanisms of tissue-selective drug toxicity: effects of liver-specific knockout of the NADPH-cytochrome P450 reductase gene on acetaminophen toxicity in kidney, lung and nasal mucosa. Mol. Pharmacol., 67: 623-630. DOI: 10.1124/mol.104.007898

Habig, W.H., M.J. Pabst and W.B. Jakpoly, 1974. Glutathione S-transferases. The first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139. PMID: 4436300

Hai-Liang, X., X. Yan-Feng, Y.U.E. Xiao-Qiang, H. Yin-Huan and L. Min *et al*., 2008. Analysis of chemical constituents in extract from *Portulaca oleracea* L. with GC-MS Method. Pharmaceut. J. Chin. People's Liberat. Army.

Jin, Z., L. Jiao, L. Jian-hua, M. Chun-yue and Z. Li-dan *et al*., 2007. The extraction and identification of the total flavanone of baise's purslane by ultrasonic wave. Lishizhen Med. Mater. Med. Res.

James, L.P, P.R. Mayeux and J.A. Hinson, 2003. Acetaminophen-induced hepatotoxicity. Drug Metab. Dispos., 31: 1499-1506. DOI: 10.1124/dmd.31.12.1499

Klein, B., P.A. Read and L.A. Babson, 1960. Rapid method for the quantitative determination of serum alkaline phosphatase. Clin. Chem., 6: 269-275. PMID: 14409655

Knight, T.R., A. Kurtz, M.L. Bajt, J.A. Hinson and H. Jaeschkae, 2001. Vascular and hepatocellular peroxynitrite formation during acetaminophen toxicity: Role of mitochondrial oxidant stress. Toxicol. Sci., 62: 212-220. DOI: 10.1093/toxsci/62.2.212

Kulkarni, A.S., B.D. Siraskar, A.D. Deshpande, A.V. Kulkarni and S.M. Dhonde *et al*., 2007. Study of hepatoprotective activity of *Portulaca oleracea* L. on rifampicin induced hepatotoxicity in rat. Pharmacist., 2: 1-5.

Lowry, O.H., N.J. Rosenbrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275. PMID: 14907713

Mansour, H.H., H.F. Hafez and N.M. Fahmy, 2006. Silymarin modulates Cisplatin-induced oxidative stress and hepatotoxicity in rats. J. Biochem. Mol. Biol., 39: 656-661. PMID: 17129399

Maryann, M. and P. Jeanmarie, 2008. Acetaminophen-induced nephrotoxicity: Pathophysiology, clinical manifestations and management. J. Med. Toxicol., 4: 2-6. DOI: 10.1007/BF03160941

Michael, S.L., N.R. Pumford, P.R. Mayeux, M.R. Niesman and J.A. Hinson, 1999. Pretreatment of mice with macrophage inactivators decreases acetaminophen hepatotoxicity and the formation of reactive oxygen and nitrogen species. Hepatol., 30: 186-195. DOI: 10.1002/hep.510300104

Mitchell, J.R., D.J. Jollow, W.Z. Potter, J.R. Gillete and B.B. Brodie, 1973a. Acetaminophen-induced hepatic necrosis. IV. Protective role of glutathione. J. Pharmacol. Exp. Ther., 187: 211-217. PMID: 4746329

Mitchell, J.R., D.J. Jollow, W.Z. Potter, D.C. Davis and J.R. Gillette *et al*., 1973b. Acetaminophen-induced hepatic necrosis. I. Role of drug metabolism. J. Pharmacol. Exp. Ther., 187: 185-194. PMID: 4746326

Montilla, P., M. Barcos, M.C. Munoz, I. Bujalance and J.R. Munoz-Castaneda *et al*., 2005. Red wine prevents brain oxidative stress and nephropathy in streptozotocin-induced diabetic rats. J. Biochem. Mol. Biol., 38: 539-544. PMID: 16202232

Moss, D.W. and P.J. Butterworth, 1974. Enzymology and medicine. 1st Edn., Pitman Medical, London, pp: 175.

Muriel, P., T. Garciapiña, V. Perez-Alvarez and M. Mourelle, 1992. Silymarin protects against paracetamol-induced lipid peroxidation and liver damage. J. Appl. Toxicol., 12: 439-442. DOI: 10.1002/jat.2550120613

Nakae, D., K. Yamamoto, H. Yoshij, T. Kinugasa and H. Marayuma *et al*., 1990. Liposome-encapsulated superoxide dismutase prevents liver necrosis induced by Acetaminophen. Am. J. Pathol., 136: 787-795. PMID: 2158237

Navarro, V.J. and J.R. Senior, 2006. Drug-related hepatotoxicity. N. Engl. J. Med., 354: 731-739. DOI: 10.1056/NEJMra052270

Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358. DOI: 10.1016/0003-2697(79)90738-3

Okwuasaba, F., C. Ejike and O. Parry, 1987. Skeletal muscle relaxant properties of the aqueous extract of Portulaca oleracea. J Ethnopharmacol., 17: 139-160. PMID: 3796017

Yemitan, O.K. and M.C. Izegbu, 2006. Protective effects of *Zingiber officinale* (Zingiberaceae) against carbon tetrachloride and acetaminophen-induced hepatotoxicity in rats. Phytotherap. Res., 20: 997-1002. DOI: 10.1002/ptr.1957

Paglia, D.E. and W.N. Valentine, 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J. Lab. Clin. Med., 70: 158-169. PMID: 6066618

Parry, O., F.K. Okwuasaba and C. Ejike, 1987. Skeletal muscle relaxant action of an aqueous extract of Portulaca oleracea in the rat. J. Ethnopharmacol., 19: 247-253. DOI: 10.1016/0378-8741(87)90002-X

Parry, O., J.A. Marks and F.K. Okwuasaba, 1993. The skeletal muscle relaxant action of *Portulaca* oleracea: role of potassium ions. J. Ethnopharmacol., 40: 187-194. DOI: 10.1016/0378-8741(93)90067-F

Proudfoot, A.T. and N. Wright, 1970. Acute Paracetamol poisoning. Br. Med. J., 3: 557-558.

Sarsilmaz, M., A. Songur, I. Kus, B. Ozyurt and M. Gulec *et al*., 2003. The regulatory role of dietary ω-3 essential fatty acids on oxidant/antioxidant balance in rat hippocampus. Neurosci. Res. Commun., 33: 114-123. DOI: 10.1002/nrc.10087

Sener, G., G.Z. Omurtag, O. Sehirli, A. Tozan and M. Yuksel *et al*., 2006. Protective effects of Ginkgo biloba against acetaminophen-induced toxicity in mice. Mol. Cell. Biochem., 283: 39-45. DOI: 10.1007/s11010-006-2268-8

Sener, G., A.Ö. Sehirli and G. Ayanoğlu-Dülger, 2003. Protective effects of melatonin, vitamin E and N-acetylcysteine against acetaminophen toxicity in mice: A comparative study. J. Pineal. Res., 35: 61-68. DOI: 10.1034/j.1600-079X.2003.00050.x

Seven, A., S. Güzel, O. Seymen, S. Civelek and M. Bolayirh *et al*., 2004. Effects of vitamin E supplementation on oxidative stress in streptozotocin induced diabetic rats: Investigation of liver and plasma. Yonsei Med. J., 45: 703-710. PMID: 15344213

Shahjahan, M., K.E. Sabithak, M. Jainu and C.S.S. Devi, 2004. Effect of Solanum trilobatum against carbon tetrachloride induced hepatic damage in albino rats. Indian J. Med. Res., 120: 194-198. PMID: 15489557

Shakun, N.P. and I.I.U. Vysotski, 1982. Effect of tetracycline antibiotics on lipid peroxidation. Antibiotiki., 27: 684-687. PMID: 7149691

Simopoulos, A.P., H.A. Norman and J.E. Gillaspy, 1995. Purslane in human nutrition and its potential for world agriculture. World Rev. Nutr. Diet., 77: 47-74. PMID: 7732700

Sinha, A.K., 1972. Colorimetric assay of catalase. Ann. Biochem., 47: 389-394. DOI: 10.1016/0003-2697(72)90132-7

Thomas, S.H.L., 1993. Paracetamol (Acetaminophen) poisoning. Pharmacol. Ther., 60: 91-120. DOI: 10.1016/0163-7258(93)90023-7

Yang, Z., C. Liu, L. Xiang and Y. Zheng, 2009. Phenolic alkaloids as a new class of antioxidants in Portulaca oleracea. Phytother. Res., 23: 1032-1035. DOI: 10.1002/ptr.2742

Zeng, X., 1999. A study of scavenging action of purslane aquenous extracts on oxygen free radical. Hunan Yi Ke Da Xue Xue Bao., 24: 133-135. PMID: 11938770

Zhang, X.J., Y.B. Ji, Y. Qu Zh, J.Ch. Xia and L. Wang, 2002. Experimental studies on antibiotic functions of *Portulaca oleracea* L. Vitro. Chin. J. Microbiol., 14: 277-280.