Biochemical study on rule of Sonchus oleraceus L. Extracts against Paracetamol-Induced Liver toxicity

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Abstract

Sonchus oleraceus L. extracts (ethanolic and aqueous) were evaluated for hepatoprotective activity against liver toxicity induced by paracetamol. Oral administration of both extracts at a concentration of 150 mg/kg b.w daily for 15 days showed a significant protection against-induced alteration in aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities and concentrations of thiobarbituric acid reactive substances (TBARS), protein thiols (Pr-SHs), reduced glutathione (GSH), by paracetamol. The results from the present investigation were supported by histopathological findings that demonstrated regeneration of hepatocytes in treated animals, which was indicative of the protective effects of the extracts. Thus the results obtained indicate that (ethanolic and aqueous) extracts of Sonchus oleraceus L. possess hepatoprotecive effect and these explicate justification of the use of this plant in the treatment of oxidative stress disease conditions.

Introduction

Paracetamol is a safe and effective analgesic/antipyretic drug when used at therapeutic levels (1). However, paracetamol overdose led to hepatotoxicity with several cases of cirrhosis, hepatitis and suicide attempts (2). Paracetamol is directly conjugated with glucuronic acid or sulphate through glucuronyl transferases or sulfonyltransferases and excreted into the bile by multidrug resistance associated protein (Mrp2) or into the blood by Mrp3. The remaining unconjugated paracetamol is then metabolized by P450 enzymes into N-acetyle P-benzoquinone imine (NAPQI), the most relevant isoenzyme being CYP2E1, especially in humans (3). On the other hand, antioxidants have been tested as alternative treatment against paracetamol toxicity (4). Oxidative stress is mainly regulated by the cellular enzymatic (catalase, superoxide dismutase, glutathione peroxidase activities) and nonenzymatic (glutathione, ascorbic acid, α-tocopherol levels) factors (5). Many studies have shown that natural antioxidants obtained from different alternative systems of medicine display a wide range of biological activities.Various alternatives possessing antioxidant properties have been used in order to minimize paracetamol induced oxidative stress in animal models. Many plant extracts have been reported to be effective in ameliorating organ toxicities (6-9). Amoung of these plants; Sonchus oleraceus L. (Compositae), an herb native to Europe, North Africa, and Asia, is widely found in moist areas of fields, orchards, roadsides, gardens, or cleared land (10). This plant has been used in folk medicines to treat diseases such as enteritis, diarrhea, pneumonia, hepatitis, appendicitis, chronic bronchopneumonia, icterus, throat swelling, haematemesis and uraemia (11). Not surprisingly, plants such as *Sonchus oleraceus L.* contain high levels of poly-phenols polyphenolics such as rutin, quercetin, catechin and myricetin (12), luteolin and apigenin (13), fatty acids, phenolic acids (14), volatile essential oils (15) and terpenes (16), which

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are excellent scavengers of reactive and represent a promising hepatopratective effect against paracetamol-induced liver toxicity. *In vivo* and *in vitro* tests have been conducted with *Sonchus oleraceus L.* to determine, for example, it's antiinflammatory and antioxidant activity (17, 18). But there are no reports on the effect of *Sonchus oleraceus L.* on liver damage. This study aims to investigated the hepatoprotective activities of the ethanolic and aquouse extracts of *Sonchus oleraceus L.* by assessing the changes in several serum and liver enzymes using paracetamol to induce acute hepatotoxicity in rats.

Materials and Methods

Materials

Chemicals:

- Paracetamol was provided as gift from El-Nile Pharmaceutical Company (Cairo, Egypt). When intended to be used in vivo experiments, paracetamol was suspended in 0.5 % tween 80 and orally administrated in dose of 1g/kg.B.W.(19).
- Tween 80 was produced by Prolabo, Farance.

Plant material:

Whole plant (leaves, stem, flowers, seeds and roots) of Sonchus oleraceus L. at maturity was collected from farms of Horbite Village, Sharkia, Egypt.

Extracts:

Preparation of ethanolic extract:

Air-dried leaves of the plant (1.5 kg) was crushed to coarse powder and extracted exhaustively in a Soxhlet with 95% ethanol. The extract was concentrated under reduced pressure to yield viscous mass. The ethanolic extract was kept in airtight containers in a deep freeze maintained at 40 ^{o}C until the time of further use.

Preparation of aqueous extract:

The aqueous extract of air-dried leaves of the plant was prepared by dissolving a known amount of air-dried leaves powder in distilled water using a magnetic stirrer. It was then filtered and evaporated to dryness under reduced pressure. An aqueous suspension, which is the form customarily, used in folk medicine, was prepared to facilitate easy handling. The drug solutions were prepared freshly each time and administered intragastrically.The dosage schedule for the drug was once a day.

Phytochemical screening:

A phytochemical analysis of aerial parts of Sonchus oleraceus L. was conducted for the detection of alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, volatile oil and triterpenes (20).

Animals:

Adult albino rats weighing around 200-220gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were acclimatized to animal house conditions. Animals were provided with standard diet and water adlibtum. Rats were kept under constant environmental condition and observed daily throughout the experimental work.

Experimental set up:

This experiment was carried out to examine the prophylactic potential of ethanolic and aqueous extracts of Sonchus oleraceus L. leaves, gaven repeatedly for 2 weeks, against paracetamol hepatotoxicity in vivo.

Groups of animals each consisting of 8 rats were treated daily for 14 days as follows. A suspended solution of 3g % was prepared for intragastric intubation of rats.

Group I: Normal (was given similar volume of tween 80 1% orally)

Group II: Control (was given similar volume of saline orally)

Group III: Was treated with ethanolic extract (150mg/kg b.w.) suspended in tween 80 orally in a single daily dose (21).

Group IV: Was treated with aqueous extract (150 mg/kg b.w.) dissolved in saline orally in a single daily dose (21).

Group V: Was treated with vitamin C (1g/kg b.w.) suspended in tween 80 orally in a single daily dose (22)**.**

At day 13, i.e. one day before the last treatment, animals of all groups were fasted for 18 h. At day 14, one hour after the last dose of drug treatment, all animals in groups II, III, IV and V recived paracetamol (1 g/kg.b.w.).

I-Treatment of blood samples

After 15 days of treatment blood samples were withdrawn from the retro-orbital vein of each animal and each sample was collected into 2 tubes, heparinized and non-heparinized. The non heparinized blood samples were allowed to coagulate and then centrifuged at 1000 xg for 20 min. The separated sera were used for the estimation of serum activity of ALT, AST, ALP, LDH as well as levels of TBARS, Pr-SHs and total protein. The heparinized blood samples were divided into 2 aliquots. The first aliquot was used for determination of GPx activity.

The second aliquot was haemolyzed using bidistilled water and the haemolysate of each sample was divided into two portions was treated with chloroform/ethanol (3:5 V/V) mixture to

precipitate and the resultant supernatant was used for the determination of SOD activity. The second portion was deproteinized with meta-phosphoric acid and the clear supernatant was used for the estimation of GSH level. Haemoglobin levels were determined in the heparinized blood samples and used in the calculation of the enzyme activity.

II-Preparation of liver samples

Animals were killed by cervical dislocation, and then livers were rapidly removed. A part of each liver was weighed and homogenized, using glass homogenizer (Universal Lab. Aid MPW-309, mechanika precyzyjna, Poland), with ice-cooled saline to prepare 25% W/V homogenate. The homogenate was divided into three aliquots. The first one was deproteinized with ice-cooled 12% trichloroacetic acid and the obtained supernatant, after centrifugation at 1000 xg, was used for the estimation of GSH. The second aliquot was centrifuged at 1000 xg and the resultant supernatant was used for estimation of LDH activity, TBARS, Pr-SHs, total protein and albumin levels. The third aliquot of homogenate was used to prepare a cytosolic fraction of the liver by centrifugation at 10500 xg for 15 min at 4 ^{0}C using a cooling ultra-centrifuge (Sorvall comiplus T-880, Du Pont, USA), and the clear supernatant (cytosolic fraction) was used for the determination of SOD and GPx activities.

Biochemical assays:

Analysis of serum was carried out for transaminases (L-alanine and L-aspartate) (23), alkaline phosphatase (ALP) (24), serum and hepatic LDH, TBARS, Pr-SHs and GSH levels in blood and hepatactivic were done by the methods described by Buhl and Jackson (25), Uchiyama and Mihara (26), Koster, et al.,(27) and Chanarin (28), respectively. Blood and liver Superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities were carried out Paglia and Valentine (29), Marklund and Marklund (30), respectively. Liver GSH were estimated according to the method of Sedlak and Lindsay (31). Blood haemoglobin was determined according to the method of van Kampen and Zijlstra (32). The protein content of liver tissue was measured by applying the method of Lowry et al. (33).

Histopathology:

The liver tissues isolated from the test animals were fixed in formaline-saline for 48 hours. The fixed tissue were processed manually through graded ethanol, cleared in xylene, impregnated and embedded in paraffin wax. Thin sections were cut with a rotary microtome, stained by haematoxylin and eosin technique, examined microscopically for pathological changes according to the method of Bancroft and Steven (34).

Statistical Analysis:

All the grouped data were statistically evaluated with SPSS/11 software. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by least significant difference (LSD) test. P values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SD for eight separate determinations (35)

Results

Table 1 shows the activities of serum ALT, AST and ALP of control and experimental groups of rats. Paracetamol (1 g/kg.), orally given to rats markedly increased serum ALT, AST and ALP activities. Whereas paracetamol injected rats-treated with the *Sonchus oleraceus L.* extract (ethanolic or aqueous) restored the altered values to the near normalcy. The decreased concentration of serum and hepatic Pr-SHs was observed in paracetamol injected rats. Administration of *Sonchus oleraceus L.* extract (ethanolic or aqueous) tends to bring the Pr-SHs level to near normal. Furthermore, vitamin C a proven liver protecting agent, also significantly inhibited the lipid peroxidetion of liver.

Tables 2 & 3 show serum and liver LDH activity, TBARS and Pr-SHs the concentrations of control and experimental groups of rats. Paracetamol (1 g/kg.), orally given to rats markedly increased serum and hepatic LDH activities. Also, the levels of serum and hepatic TBARS in paracetamol injected rats were significantly higher than control rats, whereas paracetamol injected rats-treated with the *Sonchus oleraceus L.* extract (ethanolic or aqueous) restored the altered values to the near normalcy. The decreased concentration of serum and hepatic Pr-SHs was observed in paracetamol injected rats. Administration of *Sonchus oleraceus L.* extract (ethanolic or aqueous) tends to bring the Pr-SHs level to near normal. Furthermore, vitamin C a proven liver protecting agent, also significantly inhibited the lipid peroxidetion of liver.

Tables 4 & 5 show the concentrations of blood and liver reduced glutathione (GSH), activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) of control and experimental groups of rats. The decreased concentration of blood and hepatic GSH was observed in paracetamol control rats. Administration of *Sonchus*

3

oleraceus L. extract (ethanolic or aqueous) tends to bring the GSH level to near normal. The activities of SOD and GPx in liver were significantly lower in paracetamol injected rats compared to paracetamol injected rats-treated with *Sonchus oleraceus L.* extract (ethanolic or aqueous). Vitamin C, on the other hand, also exhibited a significant hepatoprotective effect on the enzymes tested.

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 *Sonchus oleraceus L.* (ethanolic and aqueous (150mg/kg) + paracetamol together (Fig. 1C&D). The histological pattern was almost normal in rats treated with vitamin C+paracetamol (Fig. 1E).

Table 1: Activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP)in serum of normal and experimental groups of rats.

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal 1 % tween 80	52.45 ± 4.12	45.31 ± 4.08	187.05± 14.27
Control (Paracetamol 1 g/kg.b.w)	146.58 ± 15.63*	167.36 ±11.46*	420.09 ± 22.53*
Ethanolic extract 150 mg/kg.b.w.	58.07 ± 8.93$^@$	43.80 ± 5.28$^@$	164.57± 19.88$^@$
Aqueous extract 150 mg/kg b.w.	63.16 ± 7.52$^@$	56.00 ± 8.14$^@$	171.09± 20.44$^@$
Vitamin C 1 g/kg,b.w	67.78 ± 9.11$^@$	75.19 ± 6.84$^@$	192.50 ± 23.16$^@$

Paracetmol was given orally as a single dose of 1g/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose of each was given 1 h before paracetmol administration. Blood samples were collected 24 h after paracetamol adminstation. Values are given as mean ± SD for groups of eight animals each. * Significantly different from normal group at $p< 0.01$ $^@$ Significantly different from control group at $p< 0.05$.

Table 2: Activity of lactate dehydrogenase (LDH) and levels of lipid peroxides (TBARS) and protein thiols (Pr-SHs) in serum of normal and experimental groups of rats.

Groups	LDH (U/l)	TBARS nmol/ml	Pr-SHs µmol/l
Normal (1 % tween 80)	37.88 ± 3.25	2.38 ± 0.06	311.72 ± 17.25
Control (Paracetamol 1g/kg.b.w)	$105.22 \pm 9.43^{*}$	$5.45 \pm 2.00^{*}$	$158.94 \pm 20.18^{*}$
Ethanolic extract 150 mg/kg.b.w.	$35.97 \pm 8.15^{@}$	$1.75 \pm 0.36^{@}$	$297.52 \pm 15.46^{@}$
Aqueous extract 150 mg/kg b.w.	$52.24 \pm 6.11^{@}$	$3.66 \pm 0.67^{@}$	$225.47 \pm 13.05^{@}$
Vitamin C (1g/kg.b.w.)	$65.17 \pm 11.24^{@}$	$2.23 \pm 0.81^{@}$	$255.80 \pm 17.44^{@}$

Paracetamol was given orally as a single dose of 1g/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose of each was given 1 h before parcetamol administration. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at $p < 0.01$.   @ Significantly different from control group at $p < 0.05$.

Table 3: Activity of lactate dehydrogenase (LDH) and levels of lipid peroxides (TBARS) and protein thiols (Pr-SHs) in liver of normal and experimental groups of rats.

Groups	LDH (U/g protein)	TBARS nmol/g protein	Pr-SHs µmol/g protein
Normal 1 % tween 80	105.42 ± 8.32	310.95 ± 22.48	57.23 ± 43
Control (Paracetamol 1 g/kg.b.w)	$322.77 \pm 19.04^{*}$	$505.36 \pm 26.57^{*}$	$21.66 \pm 3.72^{*}$
Ethanolic extract 150 mg/kg.b.w.	$127.16 \pm 16.56^{@}$	$324.22 \pm 19.05^{@}$	$61.57 \pm 7.88^{@}$
Aqueous extract 150 mg/kg b.w.	$142.33 \pm 20.84^{@}$	$377.48 \pm 15.21^{@}$	$52.09 \pm 11.40^{@}$
Vitamin C 1 g/kg.b.w.	$115.73 \pm 13.28^{@}$	$302.69 \pm 17.55^{@}$	$69.13 \pm 8.05^{@}$

Paracetamol was given orally as a single dose of 1 g/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose of each was given 1 h before paracetamol administration. Livers were isolated 24 h after paracetamol administration. LDH activity was determined in liver cytosols while TBARS and Pr-SHs were determined in liver homogenate. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at $p < 0.01$.   @ Significantly different from control group at $p < 0.05$.

Table 4: Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in blood of normal and experimental groups of rats.

Groups	GSH (mg %)	SOD (U/g Hb)	GPx (U/g Hb)
Normal (1 % tween 80)	32.45 ± 6.10	17.80 ± 3.57	104.75 ± 11.69
Control (Paracetamol 1 g/kg.b.w)	$17.66 \pm 4.40^*$	$10.00 \pm 2.71^*$	$63.18 \pm 9.06^*$
Ethanolic extract 150 mg/kg.b.w.	$29.80 \pm 6.08^{@}$	$19.35 \pm 4.00^{@}$	$118.80 \pm 13.42^{@}$
Aqueous extract 150 mg/kg b.w.	$21.37 \pm 5.10^{@}$	$14.53 \pm 3.84^{@}$	$95.16 \pm 8.43^{@}$
Vitamin C 1 g/kg,b.w	$25.03 \pm 2.94^{@}$	$15.27 \pm 2.11^{@}$	$100.50 \pm 12.72^{@}$

Paracetmol was given orally as a single dose of 1g/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose of each was given 1 h before paracetmol administration. Blood samples were collected 24 h after paracetamol adminstation. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the haemoglobin (Hb) concentration. Values are given as mean $\pm$ SD for groups of eight animals each. * Significantly different from normal group at *p< 0.01*. @ Significantly different from control group at *p< 0.05*.

Table 5: Level of reduced glutathione (GSH) and activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) in liver of normal and experimental groups of rats.

Groups	GSH (mg/mg protein)	SOD (U/mg protein)	GPx (U/mg protein)
Normal 1 % tween 80	5.70 ± 0.087	11.82 ± 2.09	9.28 ± 1.33
Control (Paracetamol 1g/kg.b.w.)	$3.45 \pm 0.09^*$	$6.70 \pm 1.46^*$	$4.80 \pm 1.20^*$
Ethanolic extract 150 mg/kg.b.w.	$5.10 \pm 0.73^{@}$	$10.92 \pm 3.11^{@}$	$8.17 \pm 2.31^{@}$
Aqueous extract 150 mg/kg b.w.	$4.89 \pm 0.60^{@}$	$8.06 \pm 2.30^{@}$	$6.55 \pm 1.99^{@}$
Vitamin C 1g/kg.b.w.	$5.66 \pm 0.055^{@}$	$9.50 \pm 3.44^{@}$	$7.16 \pm 2.00^{@}$

Paracetamol was given orally as a single dose of 1g/kg.b.w. to 18 h fasted animals. It was given to all groups except the normal one. The test extracts and vitamin C were orally given daily for 2 weeks and the last dose of each was given 1 h before paracetamol administration. Livers were isolated 24 h after paracetamol administration. GPx and SOD activities were determined in liver cytosols while GSH was determined in liver homogenate.. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration. Values are given as mean $\pm$ SD for groups of eight animals each. * Significantly different from normal group at *p< 0.01*. @ Significantly different from control group at *p< 0.05*.
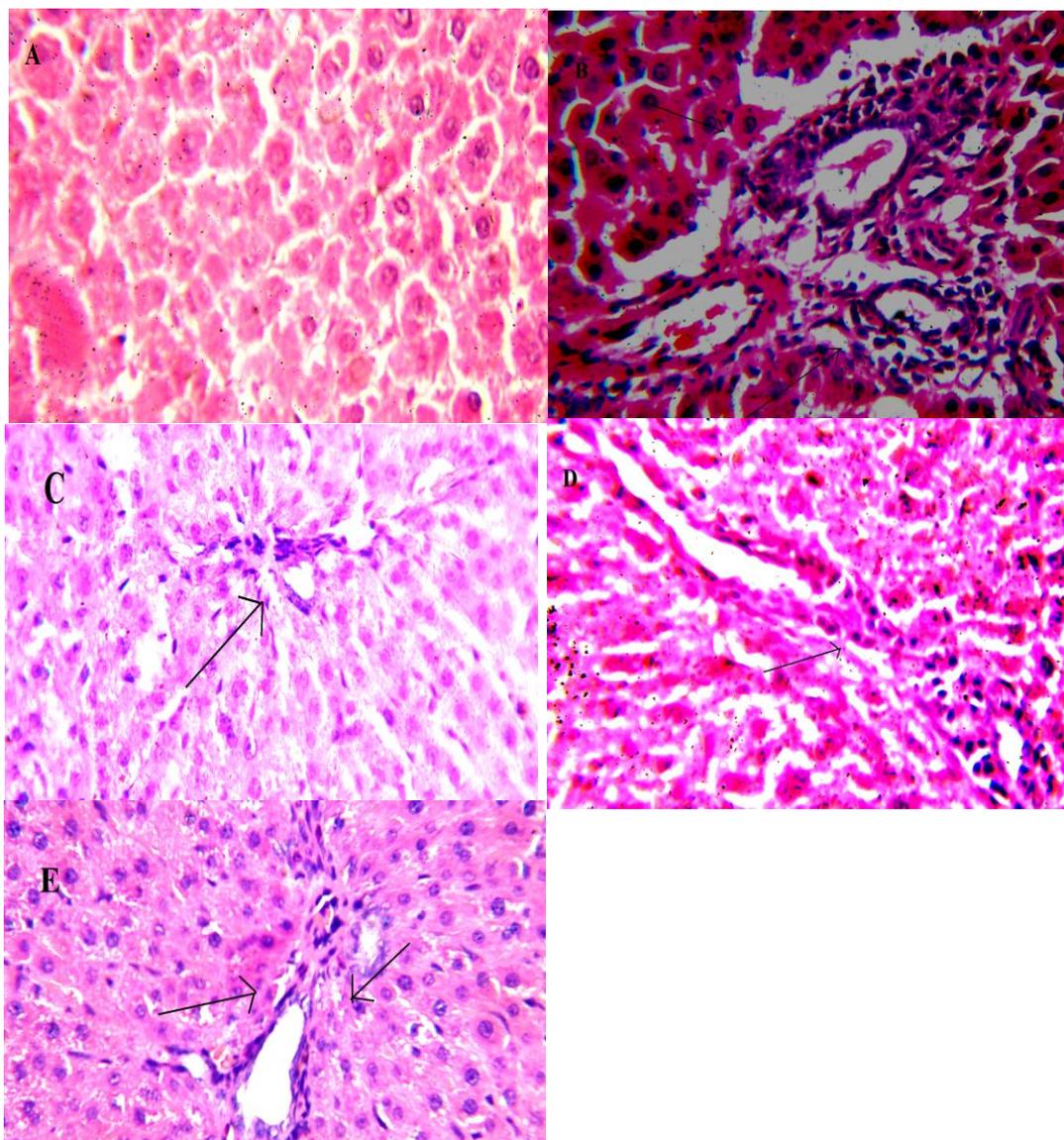
Fig.1. Representative photographs from the liver showing the protective effect of *Sonchus oleraceus L.* on paracetamol-induced hepatic injury in rats. (A), Control rat liver. Normal hepatic parenchyma; (B), Paracetamol-treated rat liver showing cloudy swelling, fatty degeneration of hepatocytes, with necrosis, heavy haemorrhage, irregular appearance and damaged central vein; (C and D) *Sonchus oleraceus L.* ethanolic and aqueous (150mg/kg) + paracetamol-treated rat liver. Normal appearance of hepatocytes with mild sinusoidal dilation; (E) Vitamin C (1g/ kg) + paracetamol treated rat liver showing near-normal appearance of hepatocyte surround the central vein.

Discussion

Paracetamol (4'-Hydroxyacetanilide) is oral analgesic and antipyretic drug (36). It is metabolized extensively by the liver *via* three main pathways; sulfonation, glucuronidation and oxidation (37). The first two pathways are quantitatively more important than the last, but the oxidative pathway is the culprit as far as toxicity is concerned (38). Oxidation of paracetamol occurs in the hepatic microsomes and is primarily catalyzed by cytochrome P-450 (39). The process produces a highly reactive arylating compound called N-acetyl-p-benzoquinoneimine (NAPQI) (40). In human liver microsome P-4501A2, were shown to be principal catalysts of paracetamol activation (41).When more NAPQI if formed than can be conjugated to GSH, the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins (42).

In the present study administration of paracetamol treated rats showed an increase in the activities of AST, ALT, ALP, LDH and TBARs levels, however there was a decrease of Pr-SHs levels besides GSH, SOD and GPx activities when compared with control rats. Oral administration of ethanolic and aqueous extracts of *Sonchus oleraceus L.* (150mg/kg body weight) and vitamin C to paracetamol treated rats showed an inhibition in activities of serum AST, ALT, ALP and LDH as well as reduction of Pr-SHs, GSH, SOD and GPx levels than paracetamol alone treated rats. Mitra *et al.*, (43) have reported that administration of paracetamol caused significantly increased serum AST and ALT activities.

The preliminary phytochemical screening showed that the ethanolic and aqueous extracts of *Sonchus oleraceus L.* contained tannins, reducing sugars, sterols, alkaloids, cardiac glycosides, flavonoids, tannins, anthraquinones, saponins, β-carotene, volatile oil and triterpenes. This is in confirmation to study done by Florence et al., (20). The presence of many biologically active phytochemicals such as triterpenes, flavonoids, alkaloids, steroids, tannins and glycosides in various plant extracts may be responsible for their respective pharmacological properties (12-16). Kumar *et al.*, (44) reported that the administration of β-carotene to paracetamol treated rats decreases the elevated activities of serum liver marker enzymes.

In the present study, oral administration of both extracts at a concentration of 150 mg/kg b.w daily for 15 days showed a significant hepatoprotecive effect. The effect was more pronounced in ethanolic extract compared aqueous extract. Large amounts of phenolic compounds, (coumarins, flavonoids, alkaloids, saponins (20) in ethanolic extract may contribute towards the antioxidant properties.

The preliminary studies conducted by this work revealed the non-toxic nature of *Sonchus oleraceus L.* on normal rats. Hepatic necrosis following massive paracetamol administration is well documented (45). Drastic elevation in the activity of liver ALT, AST, ALP and LDH were shown in the current study after administration of paracetamol (1g/kg.b.w) due to the intracellular accumulation of Ca^{2+}, which results in activation of phosphofructokinase and anaerobic glycolysis leading to lactate formation (46). Loss of Ca^{2+} homeostasis as a result of oxidative damage and increase in intracellular Ca^{2+} has been reported to be late and perhaps irreversible final stage in the process of cell death for paracetamol (47). *Sonchus oleraceus L.* (ethanolic and aqueous) administration controled serum and hepatic LDH activities .

In the present study, serum and hepatic TBARS levels were significantly lower in the *Sonchus oleraceus L.* extracts–treated groups compared to the paracetamol treated group. The above result suggests that the *Sonchus oleraceus L.* extracts (ethanolic and aqueous) may exert antioxidant activities and protect the tissues from lipid peroxidation. The protective effect due to treatment with *Sonchus oleraceus L.* extracts strongly indicated the possibility of the extracts being able to prevent and/or mitigate any leakages of marker enzymes into circulation, conditions the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and hence restores these enzymes activities (48).

Serum and hepatic protein thiols (Pr-SHs) contents were markedly decreased after paracetamol administration, as shown in the current investigation. The loss of Pr-SHs is held to be a critical event in the genesis of lethal injury by an acute oxidative stress (49). Such depletion is presumed to be a direct oxidation of the thiol groups of contiguous amino acids with the formation of protein-protein disulphides (49). The cytotoxic effects of paracetamol have been attributed to depletion of Pr-SHs level (50). In addition, the metabolism of paracetamol also generates GSSG, a product that reacts with Pr-SHs to form GSH mixed disulphides (50). In the present study, the elevation of Pr-SHs levels in serum and liver was observed in the *Sonchus oleraceus L.* extract-treated rats. This indicates that the *Sonchus oleraceus L.* can either increase the biosynthesis of Pr-SHs or reduce the oxidative stress.

GSH has a multifactorial role in antioxidant defense. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (51). **Liu *et al.,* (**52), suggested that the decrease in blood and liver GSH level could be the result of decreased synthesis or increased degradation of GSH by oxidative stress and tissue injury. Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased hepatic GSH content. In the present study, the elevation of GSH levels in blood and liver was observed in the *Sonchus oleraceus L.* extracts - treated rats. This indicates that the *Sonchus oleraceus L.* extracts (ethanolic and aqueous) can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects.

SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce H_2O_2 and molecular oxygen (53), hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by H_2O_2 or by glycation of enzymes (54). The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide (55). Thus, the increase in SOD activity may indirectly play an important role in the activity of catalase.

GPx plays a primary role in minimizing oxidative damage. Glutathione peroxidase (GPx), an enzyme with selenium and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of H_2O_2 or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione (56).

Reduce activities of SOD and GPx in blood and liver have been observed in paracetamol-treated rats. Several authors reported the decrease in SOD and GPx activities in paracetamol-treated animals (57, 58).

The results indicates that the *Sonchus oleraceus L.* extracts (ethanolic and aqueous) can either increase the biosynthesis of SOD and GPx or reduce the oxidative stress leading to less degradation of SOD and GPx, or have both effects. The effect was more pronounced in ethanolic extract compared to aqueous extract.

The histopathological study of liver tissues of animals is related to their function. The paracetamol hepatotoxicity presents as centrilobular necrosis (59). The histopathological studies support the biochemical findings. The paracetamol treated rats showed fatty changes, necrosis, vacuoles, space formation and loss of cell boundaries in liver. Oral administration of *Sonchus oleraceus L.* extracts (ethanolic and aqueous) (150 mg/kg body weight) and vitamin C to paracetamol treated rats brought back the above-mentioned changes in liver to near normal Coen *et al*., (60) reported that marked changes in liver such as vacuolated hepatocytes, necrosis and congested sinusoids in paracetamol treated rats. Oliveira *et al*., (61) have reported that administration of α- and β-amyrin to paracetamol treated rats liver showed normal histoarchitecture.

The preliminary phytochemical screening of *Sonchus oleraceus L.*, revealed the presence of flavonoids. Flavonoids (or bioflavonoids) are natural products, they are capable of modulating the activity of enzymes (SOD and GPx) and affecting the behavior of many cell systems and possess a significant antihepatotoxic, antiallergic, anti-inflammatory, antiosteoporotic, and even antitumor and antioxidant activities (62, 63).

In conclusion, the results of this study demonstrated that *Sonchus oleraceus L.* extracts possesses a potent hepatoprotective action upon paracetamol-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.

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