

Phytoconstituents and Hepatoprotective Effect of *Suaeda monoica* Forssk and *Suaeda pruinosa* Lange

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The objective of this study is to investigate the chemical constituents of *Suaeda monoica* and *Suaeda pruinosa* and evaluate their effects on rat liver intoxicated by over dose of paracetamol. Qualitative analysis of (80%) methanol in water fraction revealed the presence of alkaloids, terpenoids, steroids, tannins, quinones, saponins, flavonoids and phenols from two mangrove species of *S. monoica* and *S. pruinosa* was carried out. The compounds were identified from the 80% methanol-H₂O fraction as Rutin, quercetin, Syringic acid, Caffeic acid, Catechin, Coumaric acid, Vanillin, Gallic acid, Cinnamic acid. Amino acids analysis for the methanolic extract from the aerial parts of *Suaeda monoica* and *Suaeda pruinosa* showed the presence of thirteen amino acids and absence of valine, Isoleucine, Phenylalanine. Fatty acid analysis of lipids showed high percentage of long chain fatty acids. TLC of the lipoidal matter for each plant showed the presence β -amyrin, β -sitosterol and stigmaterol. Toxicity was stimulated by administration of a single oral dose of paracetamol (3 g/kg body weight). The extract of the aerial parts of plants (100 mg/kg) was utilized on a pre-and post-treatment basis. Both extracts significantly improved liver and kidney function with prophylactic or therapeutic treatments. Histopathological and histochemical studies showed parallel effects with the biochemical measured parameters.

Keywords: *Suaeda* sp.- Secondary metabolites- Paracetamol- Hepatoprotection.

A mangrove and mangrove associate plants are proved to have rich of high value secondary metabolites viz, saponins, alkaloids, polyphenols which possess antibacterial, antifungal, antiplasmodial and hepatoprotective activities¹.

Suaeda (Chenopodiaceae) is a genus of plants also known as seep weeds, includes annual and perennial herbs or rarely small trees. There are about 110 species in the *Suaeda* genus (*S.*)². They are distributed on coasts, deserts, lakeside and saline and alkaline land all over the world.

A number of species have been found to be valuable feed for livestock in arid area like *Suaeda pruinosa* Lange, *S. vera* and *S. vermiculata*³, while others have been utilized to desalinate irrigated farmlands like *Suaeda maritima*⁴. In Mexico, some species such as *Suaeda pulvinata* are cooked in traditional dishes known as romeritos. *Suaeda* species are commonly used in folk medicine⁵⁻⁶. Some species possess hypoglycemic, anti-inflammatory, hypolipidemic, cardiotoxic, antioxidant, antimicrobial and anticancer activity⁷⁻⁸.

In Saudi Arabia it is represented by 9 species according to Boulos⁹. Five species were found in Al Jouf area represented by; *S. aegyptica*, *S. vera* and *S. vermiculata*, *S. fruticosa* and *S. mollis*¹⁰.

Suaeda vera subspecies *pruinosa* (Lange), are facultative halophytes that tolerate moderate salt soils, dry soils, nitrified and saline (halonitrófilos) in very sunny places arid climate during May to October¹¹. The specific epithet “*pruinosa*” refers to the presence of pruina, which is kind of thin waxy coating on stems and leaves, which offers a glaucous appearance. In the area south of Alicante has been used to make the “*stone soda*” and was said to be as good as that made with fine barrilla (*Halogetum sativus*). The nutritive value of forage species (crude protein ranges) in *Suaeda pruinosa* Lange is 27.17%.

Suaeda monoica Forssk. ex J. F. Gmel is a salt marsh mangrove herb growing in hypersaline soils. It is smaller in size, simple leaves which are edible. Traditionally, the leaf from *S. monoica* is known to use as a medicine for hepatitis¹² and scientifically it is reported to be used as ointment for wounds¹³ and possess antiviral activity⁷ because of the presence of triterpenoids and sterols⁵.

Methanolic extracts of *S. monoica*, exhibits moderate sun protective activity and a safe, effective and affordable sun screen formulation¹⁴. Endophytic fungi were isolated from the leaves and stems of *S. monoica*, the isolation was according to the method described by Suryanarayanan *et al.*¹⁵. Phytochemical screening of methanolic extracts from *S. monoica*, showed the presence of flavonoids, tannins, phenols, polyphenols, steroids and Alkaloids¹⁶.

A polysaccharide extracted from the leaves of *S. monoica* showed positive activity against human immune deficiency viruses^{7, 13}.

Therefore, the present attempt has been made to identify the phytoconstituent of *S. monoica* and *Suaeda pruinosa* extracts and to find out the anti-inflammatory effect of each extract on the rat liver intoxicated by paracetamol overdose.

MATERIALS AND METHODS

Plant material and preparation of extract

Suaeda sp. were collected during months of June, July, 2016-2017 from El Doubia at El Riyadh- El Dallamroad. Air-dried and powdered aerial parts of *Suaeda monoica* and *Suaeda pruinosa* were extracted with petroleum ether. The solvent was evaporated under reduced pressure then extracted with methyl alcohol in H₂O(80%), the solvent was stripped off under reduced pressure gave 50 g. The petroleum ether extract 10 g was subjected to subsequent investigation.

Extract of amino acids

Lyophilized tissues were ground in a mortar and extracted with MCW (methanol: chloroform:water 12:5:3 v/v/v), following the procedure described by Marur *et al.*(1994)¹⁷. A sample of ground material (100 mg) was extracted with 10 mL of MCW for 3 days in a closed tube in a refrigerator. After centrifugation at 1200 rpm for 5 min on a bench centrifuge, the supernatant was collected and the residue extracted with a further 6 mL of MCW. After centrifugation the supernatants were combined (16mL) and shaken with chloroform (4mL) followed by water (6 mL). On standing, the resulting separation of phases enabled the removal of chlorophyll and the aqueous phase was taken to dryness under a stream of N₂ gas. This material was resuspended in water and used for amino acid determination.

Preparation of fatty acid methyl esters

This method is a modified Folch procedure¹⁸, utilizing 2:1 (v/v) chloroform/methanol as the organic solvent.

Chemicals and reagents

Amino acids analysis

Methanol (HPLC grade), Chloroform (HPLC grade) chloroform/methanol (HPLC grade) 2:1 (v/v), 0.88% (8.8 g/liter) KCl in distilled H₂O, Nitrogen gas: high purity (<5 mg O₂/kg), Centrifuge tube with spout or 50-ml glass tube, Polytron PT-3100 homogenizer equipped with a PT-DA 3012/2EC aggregate (probe) or equivalent,

Whatman no. 40 filter paper, 15- and 50-ml test tubes with Teflon-lined screw cap, Test tube shaker, Tabletop centrifuge, Vacuum aspirator, N-EVAP model 112 nitrogen evaporator (Organomation) with moisture trap in line from N₂ source, Glass pipets, Glass beakers.

Organic solvent for preparation of lipid sample

Nitrogen gas: high purity (<5 mg O₂/kg), 0.5 N NaOH: 2 g NaOH diluted to 100 ml in methanol; store up to 6 months at 2^o to 8^oC 10% to 14% boron trifluoride (BF₃) in methanol (Sigma or Supelco; also see recipe).

Isooctane or hexane, Anhydrous sodium sulfate: store at 100^oC N-EVAP model 112 nitrogen evaporator (Organomation) with moisture trap in line from N₂ source (Fig. D1.2.1), 15-ml test tubes with Teflon-lined screw caps 100^oC heating block, Test tube shaker, Tabletop centrifuge, GC sample vials with Teflon caps.

METHODS

Separation of the amino acids was carried out on a Superpac (Pharmacia) ODS-2 column coupled to an LKB dual pump HPLC system (model 2150), controlled by a gradient generator, model 2152. Knowing the concentration of amino acids in the sample, an appropriate volume was taken for the preparation of *o*-phthalaldehyde (OPA)¹⁹⁻²⁰ and 9-fluorenylmethyl-chloroformate (FMOC-Cl) derivatives²¹ prior to separation and analysis by reverse-phase HPLC. The analysis of proline was carried out by a second analysis using FMOC-Cl derivatives on each sample. FMOC derivatives were prepared as described by²².

Extracted lipid sample

Fatty acid compositional analysis of lipids carried out by gas-liquid chromatography (GLC) modified Folch procedure¹⁸.

GC method

Column: J+W DB-23 (50% cyanopropyl) methylpolysiloxane FID: Temp: 270 °C, Injection: - mode: split ratio 30:1, - temperature: 270 °C, injection volume: 1 µL
Oven: - initial temp: 140 °C, - temp ramp: 10 °C / min, - final temp: 260°, Hold at final temperature for 3 minutes. For sequences run prior to 03/30/07, the final temperature was held for 5 minutes.

HPLC conditions for phenolic compounds

HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using C18 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.02% tri-fluoro-acetic acid in acetonitrile (B) at a flow rate 1 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (80% A); 0–5 min (80% A); 5–8 min (40% A); 8–12 min (50% A); 12–14 min (80% A) and 14–16 min (80% A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 10 µl for each of the sample solutions. The column temperature was maintained at 35 °C.

Biochemical Studies

Materials and Methods

Drugs and Chemicals Paracetamol was purchased from Alexandria Company for Pharmaceuticals and Chemical Industries. Kits for determination of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), bilirubin and urea were obtained from Biomed Diagnostics, Egypt. The interleukin-1β (IL-1β) and tumor necrosis factor alpha (TNF-α) were carried out using an ELISA reagent kits obtained from Biosource, USA.

Animals

Male rats weighing 100-120 g were obtained from the Animal Breeding Lab of National Research Centre, Cairo, Egypt. The animals were kept under constant temperature conditions (22 ± 2 °C), relative humidity (50–60%), and lighting (12 h light/dark cycle). Food and water were accessible ad libitum. The study was carried out according to the guidelines of the Ethics Committee of the National Research Centre.

Experimental design

Six groups each of six male albino rats were selected. Group 1: served as negative control. Group 2: rats received a single dose of paracetamol (positive control). Group 3 & 4: rats received *Suaeda monoica* and *Suaeda pruinose* before paracetamol induction. Group 5 & 6: rats received the *Suaeda monoica* and *Suaeda pruinose* extract after paracetamol induction. Twenty-four hours after treatments, the rats of all groups were anesthetized and blood samples were collected directly from retro-orbital plexus. The blood samples were

allowed to clot for 20-30 min. Serum was separated by centrifugation at 37°C and used for estimation of various biochemical parameters. Animals were sacrificed by decapitation. Livers were rapidly isolated; a part of each was homogenized using cold saline to prepare a 10% homogenate that was used for estimation of malondialdehyde (MDA). The second part of the liver was preserved in 10% formalin for histopathological and histochemical examinations.

Biochemical analyses

The activities of AST and ALT were determined according to the method of Reitman and Frankel²³. Alkaline phosphatase was determined using the method described by Demetriou *et al.*²⁴. Bilirubin was carried out by the method described by Young²⁵. Creatinine was measured by the method of Bartels and Bohmer²⁶. Urea was calculated by the method of Tabacco *et al.*²⁷. Arylesterase activity of paraoxonase 1 (POX-1) was measured spectrophotometrically using phenylacetate as a substrate²⁸⁻²⁹. IL-1 α was carried out using an ELISA reagent kit and expressed as pg/ml. TNF- α was determined by ELISA using kit (Biosource International, USA) and microtiter plate reader (Fisher Biotech, Germany). Lipid peroxidation was assayed by measuring the level of malondialdehyde (MDA) in the tissue homogenates using the method of Ruiz-Larrea *et al.*³⁰.

Histopathological and Histochemical Studies

After the experimental period, animals were sacrificed, liver removed immediately, sliced and washed in saline. Liver pieces were preserved in 10% formalin for histopathological studies. Sections were taken and stained with hematoxylin and eosin (H&E)³¹ and then they were examined for histopathological changes and photographed. The histochemical study was performed by periodic acid-Schiff method³² for visualization of the polysaccharide in the liver. These materials were demonstrated in sections with 5 μ m thickness.

Statistical analysis

The protection percent is calculated by $1 - (T - V / C - V) \times 100$. Data were expressed as mean \pm SD. The data were analyzed by one-way ANOVA followed by Duncan's multiple tests, using SPSS software (SAS Institute Inc., Cary, NC, USA). A probability value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis

Plant materials were screened using the methods previously described³³. Phytochemical analysis gave positive test for alkaloids, terpenoids, steroids, tannins, saponins, flavonoids and phenols. The identification of major chemical groups was carried out by thin layer chromatography (TLC) on silica gel. TLC was developed in chloroform/methanol/water (26:14:3), spots were visualized with sulfuric acid 20% in methanol solution under 254 and 356 nm gave yellow spots for Flavonoids, and purple color suggested the existence of triterpene, pink color for sterols and brown color for monoterpenes. Data shown that the main compounds were observed in *S. monoica* and *S. pruinosa* phenolic and terpenes /or sterols.

Amino acids analysis

Separation of the amino acids for methanolic extracts of each plant was carried out on HPLC system revealed that, the highest percentage of Methionine in *S. pruinosa* (0.999) while *S. monoica* (0.889) and Histidine was (0.216) in *S. monoica* and (0.167) in *S. pruinosa* extracts. Valine, Isoleucine and Phenylalanine were absent from the extracts of *S. monoica* and *S. pruinosa* (table1).

GC of fatty acid methyl esters in the petroleum ether extract

Fatty acid analysis of lipids carried out by gas-liquid chromatography (GC)³⁴ showed high percentage of long chain fatty acids, the percentage of unsaturated fatty acids was (polyunsaturated fatty acids linolenic acid was (38.96%) followed by linoleic acid (59.84%) and monounsaturated fatty acid, oleic was 71.69% and the percentage of saturated fatty acids 65.26% for *S. pruinosa*, while the high percentage of linolenic acid (33.72%) followed by linoleic acid (52.42%) and oleic acid (40.71%) and 77.80 saturated fatty acids for *S. monoica* (table2).

HPLC of phenolic compounds

HPLC of phenolic compounds in Methanolic extract from the aerial parts of *S. monoica* and *S. pruinosa* revealed that, the identified compounds in methanolic extract of *S. pruinosa* were nine while methanolic extract of *S. monoica* showed eight compounds. The extract of each plant contained Gallic Acid, Catechin, Caffeic Acid, Syringic Acid, Rutin, Coumaric

Acid, Vanillin, Quercetin but cinnamic acid found in methanolic extract of *S. pruinosa* only (table 3).

Histopathological studies

At the end of the experiment, and after blood samples were obtained liver samples were taken, washed with normal saline and processed for histopathological study. Initially the materials were fixed in 10% buffered neutral formalin and paraffin sections were taken at 5 μ m thickness processed in alcohol-xylene series and was stained with alum hematoxylin and eosin, then were examined for histopathological changes.

The microscopic examination of liver of control rats shows the normal hepatic lobules

Table 1. Amino acids identified in the methanol extract from the aerial parts of each *S. monoica* and *S.pruinosa*

Identified amino acids	μ mol/mg dry wt	
	<i>S. monica</i>	<i>S. pruinosa</i>
Histidine	0.216	0.167
Arginine	0.137	0.130
Threonine	0.121	0.115
Alanine	0.091	0.084
Proline	0.082	0.077
Tyrosine	0.105	0.081
Valine	ND	ND
Methionine	0.889	0.999
Cysteine	0.151	0.143
Isoleucine	ND	ND
Leucine	0.122	0.116
Phenylalanine	ND	ND
lysine	0.312	0.297
Aspartic acid	0.111	0.086
Glutamic acid	0.035	0.027
Glycine	0.128	0.122

and portal areas structure. The central vein is surrounded by the hepatocytes with eosinophilic cytoplasm and distinct nuclei. The hepatic sinusoids are shown between the hepatocytes (Fig. 1.A, B).

Histopathological investigations of liver of rat administered a single dose of paracetamol (3 g/kg/b.w) showed widespread swelling and ballooning degeneration and focal necrosis that associated with inflammatory infiltration in the hepatocytes (Fig. 1.C). In some rats dilated and congested portal tracts (Fig. 1.D).

In rats given Sum extract and paracetamol, examination showed the hepatic lobules that appeared more or less like control (Fig. 2.A). In somerats, hydrobic degeneration and congested portal tracts were shown (Fig. 2.B). Histopathological investigation of liver of rats

Table 2. GC of fatty acid methyl esters in the petroleum ether extract from the aerial parts of *S. monoica* and *S.pruinosa*

Identified fatty acids (mg/100 g D.W)	Plant samples	
	<i>S. monica</i> Area%	<i>S. pruinosa</i> Area%
Lauric acid (C12:0)	15.78	10.49
Myristic acid(C14:0)	ND	ND
Myristoleic (C14:1)	7.55	8.27
Pentadecylic acid (C15:0)	ND	ND
Palmitic acid (C16:0)	39.41	33.39
Palmitoleic acid (C16:1)	20.65	25.17
Stearic acid (C18:0)	22.61	21.38
Oleic acid (C18:1)	40.71	38.25
Linoleic acid (C18:2)	52.42	59.84
Linolenic acid (C18:3)	33.72	38.96

Table 3. HPLC of phenolic compounds in Methanol extract from aerial parts of each *S. monoica* and *S. pruinosa*

Compounds	<i>S. monoica</i>			<i>S. pruinosa</i>		
	Area	Conc. (μ g/ml)	Conc. (μ g/g)	Area	Conc. (μ g/ml)	Conc. (μ g/g)
Gallic Acid	250.08	13.43	268.60	160.71	8.63	172.61
Catechin	132.56	25.58	511.61	155.22	29.95	599.08
Coffeic Acid	165.36	6.13	122.50	159.21	5.90	117.95
Syringic Acid	362.51	18.86	377.23	285.90	14.88	297.51
Rutin	474.19	65.43	1308.50	374.03	51.61	1032.12
Coumaric Acid	83.54	1.86	37.20	97.57	2.17	43.45
Vanillin	209.57	6.37	127.32	253.38	7.70	153.94
Quercetin	89.57	8.45	168.95	12.56	1.18	23.70
Cinnamic Acid	0.00	0.00	0.00	17.27	0.16	3.28

given Sup extract and paracetamol showed the hepatic lobules that appeared more or less like control (Fig. 2.C). In some rats hydrobic degeneration and congested portal tracts were noticed (Fig. 2.D).

Sections of liver of rats given paracetamol and Sum, and Sup extracts showed the hepatic lobules that appeared more or less like control (Fig. 3A, B). But in rats given paracetamol and Sup extract few foci of necrosis in the hepatocytes were showed (Fig. 3.B).

Examination of control liver sections stained by PAS reaction shows the abundance of glycogen in the cell of hepatic lobule. The glycogen particles appear accumulated at one side of the cytoplasm leaving the other side almost devoid of such materials (Fig. 4.A). Histochemical investigations of liver of rats administered a single dose of paracetamol dose (3 g/kg body weight) and were sacrificed after 24 h showed marked diminution in glycogen distribution in the hepatocytes as compared with control group (Fig. 4.B). Examination of sections of liver that stained with PAS in rat orally given paracetamol after 7 days of Sum and Sup plants extracts administration (prophylactic groups, 100 mg/kg b.w) indicated an increase in glycogen contents as compared with paracetamol only. The distribution of this inclusion

was more or less like normal control (Fig. 4C, D). On the other hand, in case of therapeutic groups in which paracetamol was conducted once before Sum and Sup plant extracts administration (100 mg/kg b.w) daily for 7 days showed heterogeneous distribution of glycogen, where the healthy zones exhibited normal distribution and the injured zones exhibited a decrease in this inclusion (Fig.4E, F).

Biochemical Studies

Liver regulates many important metabolic functions and contains a host of enzymes. Any injury in this organ causes distortion of these metabolic functions. The present work aimed to compare the possible hepatoprotective impact of *Suaeda* Sp. administered for one week prior to and after a single acetaminophen toxic dose (3g/kg body weight) in male rats.

The present study showed damage of liver due to paracetamol over dosage which is confirmed by elevated levels of all biochemical parameters measured (SGPT, SGOT, ALP, TB, urea, creatinine, IL1, TNF and MDA). The study also showed decrease in POX enzyme. These elevated enzymatic activities in serum, are indicators of cellular leakage and loss of functional integrity of cell membrane in liver³⁵. Aqueous extract of *S. monoica* and *S. pruinosa* contain alkaloids, terpenoids, steroids, tannins, quinones,

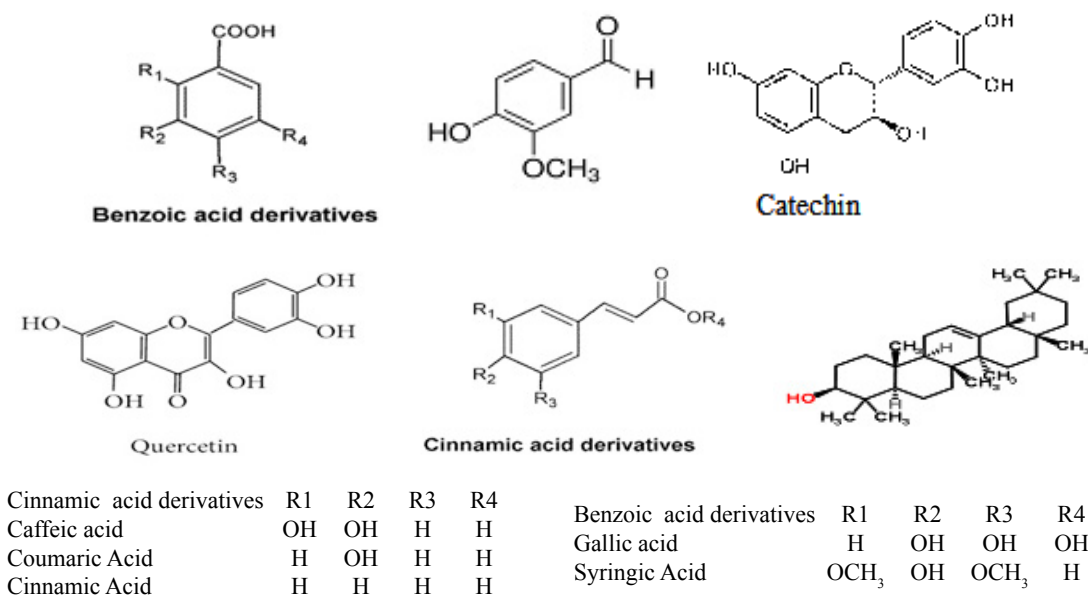


Fig. 1. Chemical Structures of compounds identified in methanolic extract from the aerial parts of *S.monoica* and *S.pruinosa*

saponins, flavonoids and phenols. They can scavenge off free radicals which are the principles for the hepatoprotective activity for protection or therapeutic effect. Decreased serum parameters level following extract treatment indicated the effectiveness of the extract in restoring normal functional status of the liver.

Suaeda had successfully reversed the PCM-induced hepatotoxic effect by its ability to reduce the elevated levels of ALT, AST, and ALP suggesting that these biochemical restorations could be due to the extract ability to inhibit the cytochrome P450 or/and ability to promote the PCM glucuronidation³⁶⁻³⁷. Furthermore, the ability to lower the enzymes level can be associated with the ability of *Suaeda* to prevent lipid peroxidation of endoplasmic reticulum that is rich in polyunsaturated fatty acid by disrupting the binding of activated radicals to the macromolecules. This

process can possibly be achieved via the antioxidant activity of *Suaeda* extracts due to the presence of high amount of α linolenic and linoleic fatty acid³⁸. Besides, mechanisms of protection that can take place include activation of liver regeneration by enhancing the protein and glycoprotein synthesis or accelerated detoxification and excretion³⁹, prevention of lipid peroxidation process, and stabilization of hepatocellular membrane³⁸. The two *Suaeda* extracts contain methionine and cysteine amino acids which act as -SH donor in synthesis of GSH and prevent APAP metabolite to interact with macromolecules. The results are confirmed with Di Pierro and Rosson,⁴⁰ who found that a mixture of L-cysteine, L-methionine, and L-serine were effective at a lower dose than N-acetyl cysteine (NAC) in APAP over dose and improved mouse survival rates. Moreover, the mixture significantly reduced ALT level and

Table 4. Effect of *Suaeda*. Sp. extract on rat liver and kidney treated with over dose paracetamol

Parameter Group name	ALT	AST	ALP	Total bilirubin	urea	Creatinine
1- Clean control	12.13± 1.17 (2,3,4,5,6)	24.74± 2.23 (2,3,4)	92.61± 2.93 (2,3,4,5,6)	0.92± 0.15 (2,3,4,5,6)	28.97± 1.95 (2,3,4,5,6)	1.15± 0.19 (2,3,4,5,6)
2- Positive control	60.11± 2.41 (1,3,4,5,6)	92.56± 2.78 (1,3,4,5,6)	261.98± 4.07 (1,3,4,5,6)	1.65± 0.18 (1,3,4,5,6)	66.49± 2.57 (1,3,4,5,6)	4.20± 0.59 (1,3,4,5,6)
3- S. monoica Pre- treated	41.21± 3.44 (1,2,4,5,6)	45.99± 2.39 (1,2,5,6)	226.39± 1.99 (1,2,5,6)	1.46± 0.03 (1,2,5,6)	52.95± 1.94 (1,2,5,6)	3.48± 0.27 (1,2,5,6)
4- S. pruinosa Pre -treated	49.07± 2.93 (1,2,3,5,6)	46.64± 2.83 (1,2,5,6)	228.89± 3.70 (1,2,5,6)	1.49± 0.03 (1,2,5,6)	55.98± 1.13 (1,2,5,6)	3.48± 0.38 (1,2,5,6)
5- S. monoica Post-treated	30.59± 2.03 (1,2,3,4)	28.44± 1.58 (1,2,3,4)	179.73± 2.72 (1,2,3,4)	1.37± 0.03 (1,2,3,4)	39.54± 2.06 (1,2,3,4)	2.29± 0.20 (1,2,3,4)
6- S. pruinosa Post-treated	31.93± 2.17 (1,2,3,4)	27.87± 2.25 (1,2,3,4)	166.62± 2.42 (1,2,3,4)	1.38± 0.05 (1,2,3,4)	39.87± 2.097 (1,2,3,4)	2.54± 0.23 (1,2,3,4)

Values represent mean of six animals ± SE

Significant change at $P < 0.05$. LSD: Least significance difference

Table 5. Effect SU. Sp. extract on antioxidant of rat treated with over dose paracetamol

Parameter Group name	1-Clean control	2- Positive control	3- S. monica Pre- treated	4- S. pruinosa Pre -treated	5- S. monica Post-treated	6- S. pruinosa Post-treated
MDA	19.91± 1.65 (2,3,4,5,6)	59.59± 2.43 (1,3,4,5,6)	48.46± 1.86 (1,2,5,6)	49.36± 1.72 (1,2,5,6)	37.09± 1.75 (1,2,3,4)	38.02± 2.09 (1,2,3,4)
POX-1	26.61± 1.70 (2,3,4,5,6)	13.18± 1.14 (1,3,4,5,6)	18.11± 1.84 (1,2)	18.54± 1.29 (1,2)	18.54± 1.29 (1,2)	18.94± 1.63 (1,2)

Values represent mean of six animals ± SE

Significant change at $P < 0.05$. LSD: Least significance difference

induced hepatic GSH synthesis and decrease MDA accumulation⁴⁰.

Increase in serum level of ALP is due to increased synthesis in the presence of increasing biliary pressure⁴¹ and reflects the pathological alteration in biliary flow⁴².

Bilirubin, a metabolic product of hemoglobin, undergoes conjugation with glucuronic acid in hepatocytes to increase its water solubility. Determination of serum bilirubin represents an index for assessment of hepatic function, and any abnormal increase in the levels of serum bilirubin indicates hepatobiliary diseases

and severe disturbance of hepatocellular function⁴³. Decreased serum bilirubin level following extract treatment indicated the effectiveness of the extract in restoring normal functional status of the liver.

Paracetamol-induced toxicity in rats may have altered membrane structure and function as well as lipids metabolism in the liver.

Therefore, it is possible to propose that the extract/compounds exerting an anti-inflammatory activity might also demonstrate hepatoprotective activity. Interestingly, this is supported by the study of Gupta *et al*⁴⁴ who suggested that the combination of hepatoprotective effect and antioxidant activity

Table 6. Effect *Suaeda*Sp. extract on immune response in rat treated with over

Parameter Group name	1-Clean control	2- Positive control	3- <i>S. monica</i> Pre- treated	4- <i>S. pruinosa</i> Pre -treated	5- <i>S. monica</i> Post-treated	6- <i>S. pruinosa</i> Post-treated
IL-1 β (Pg/ml)	29.00 \pm 1.58 (2,3,4,5,6)	93.46 \pm 2.78 (1,3,4,5,6)	77.17 \pm 1.74 (1,2,5,6)	71.55 \pm 2.49 (1,2,5,6)	54.65 \pm 2.24 (1,2,3,4)	54.89 \pm 1.92 (1,2,3,4)
TNF (Pg/ml)	17.79 \pm 1.18 (2,3,4,5,6)	45.46 \pm 1.63 (1,3,4,5,6)	38.251 \pm 1.77 (1,2,5,6)	39.53 \pm 2.08 (1,2,5,6)	30.94 \pm 1.59 (1,2,3,4)	30.44 \pm 2.38 (1,2,3,4)

Values represent mean of six animals \pm SE

Significant change at $P < 0.05$. LSD: Least significance difference

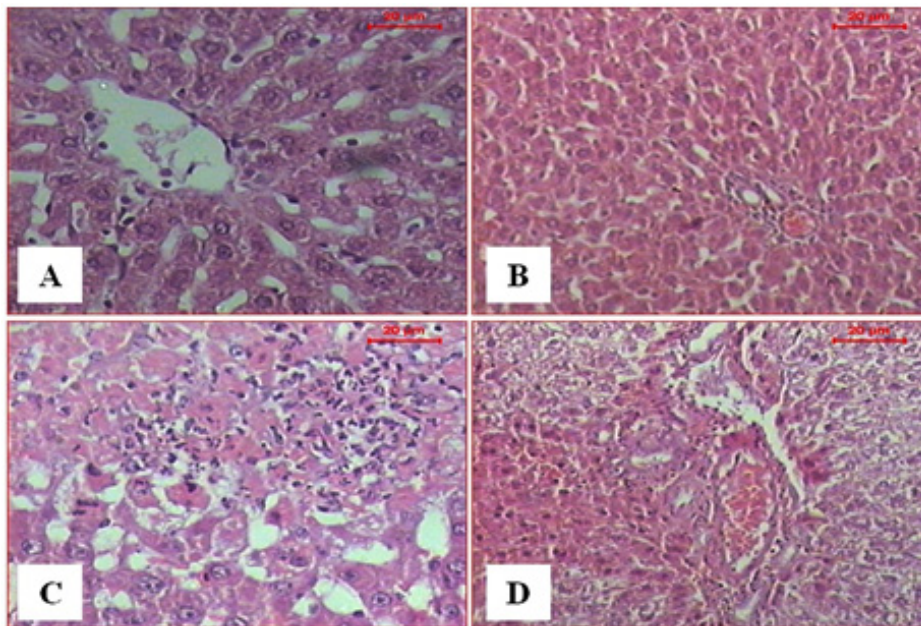


Fig. 2. Sections of liver of A, B) control rat liver shows normal structure of hepatic lobule and portal tract, respectively, C) rat administered a single dose of paracetamol (2 g/kg/b.w) shows widespread swelling and ballooning degeneration and focal necrosis that associated with inflammatory infiltration in the hepatocytes, D) rat administered a single dose of paracetamol shows the portal and periportal area with dilated and congested veins (long arrow) (H & E stain, Scale bar: 20 μ m)

synergistically prevents the process of initiation and progression of hepatocellular injury in prophylactic and therapeutic treatments⁴⁴.

Vanillin, a phenolic aldehyde has been reported to possess antioxidant and free radical scavenging ability which could possibly account for the hepatoprotective property of this plant. The activities of antioxidant counteract the redox state precipitated intracellularly and hence ensure hepatoprotection against paracetamol-induced liver injury⁴⁵⁻⁴⁷. The antioxidant activity of this extract may also explain the mechanism of the hepatoprotective activity of *Suaeda sp.* The findings of this study corroborate the effect that was reported for *Homalium zeylanicum*⁴⁸.

Lipid peroxidation (LPO) is one of the characteristic features of oxidative stress in PCM hepatotoxicity. According to Luqman and Rizvi⁴⁹, LPO is known to injure the cells by inactivation of membrane enzymes, decrease fluidity of the membrane and release, into cytotoxic, aldehydes such as MDA⁴⁹. The elevated level of hepatic MDA has been regarded as an indicator of cellular damage and indicates the inability of the antioxidant defense system to protect against the production of excessive free radicals. In this study,

MDA increased and POX decreased as a result of paracetamol over dose. The enhanced antioxidant defense in addition to reduced LPO product in the liver is indicative for the antioxidant effect of the tested extracts.

The biochemical results were supported by the histopathological findings. The results demonstrated that rats exposed to PCM alone showed severe cellular damage that might be due to the production of free radicals by PCM and subsequent LPO. These histopathologic findings were ameliorated significantly in the groups of rat wither were pre-treated or treated with either *S. monoica* or *S. peruisma* extracts, indicating pronounced protection of hepatocytes against PCM induced hepatic damage. It can be referred to the antioxidant effect of the tested extracts that markedly decreased the oxidative stress and thereby reduced the histopathological alterations of the liver.

The present study found that two Suaeda contain cysteine and Methionine with excessive amount which considered as -SH donor for GSH synthesis. Di Pierro and Rossoni⁴⁰ found that DDM-GSH (amixture of L-cysteine, L-methionine, and L-serine in a weight ratio of 2:1:1) was effective

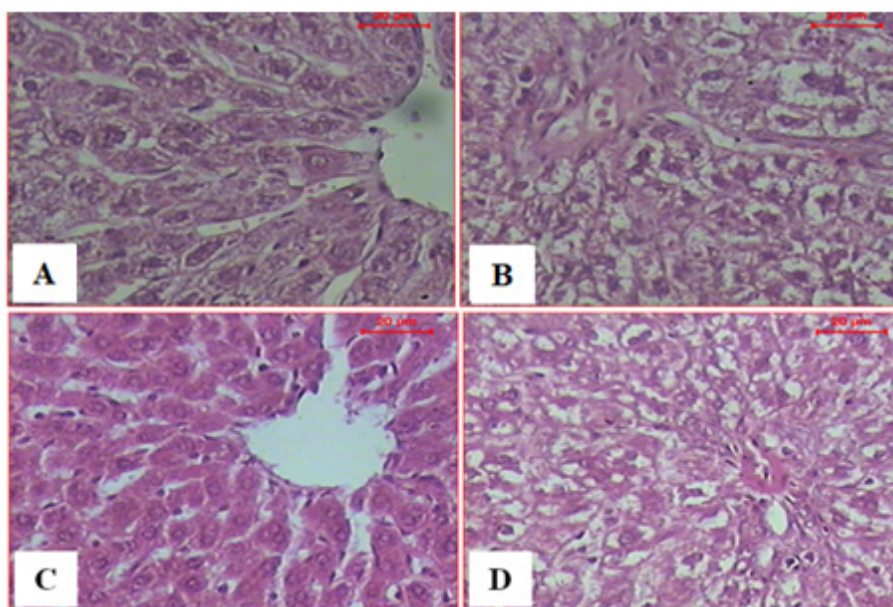


Fig. 3. Sections of liver of A)rat given Sum extract and paracetamol show the hepatic lobule that appear more or less like control, B) rat given Sum extract and paracetamol show hydrobic degeneration and congested portal tract, C)rat given Sup extract and paracetamol show the hepatic lobule that appear more or less like control, D) rat given Sup extract and paracetamol show hydrobic degeneration and congested portal tract (H & E stain, Scale bar: 20 µm)

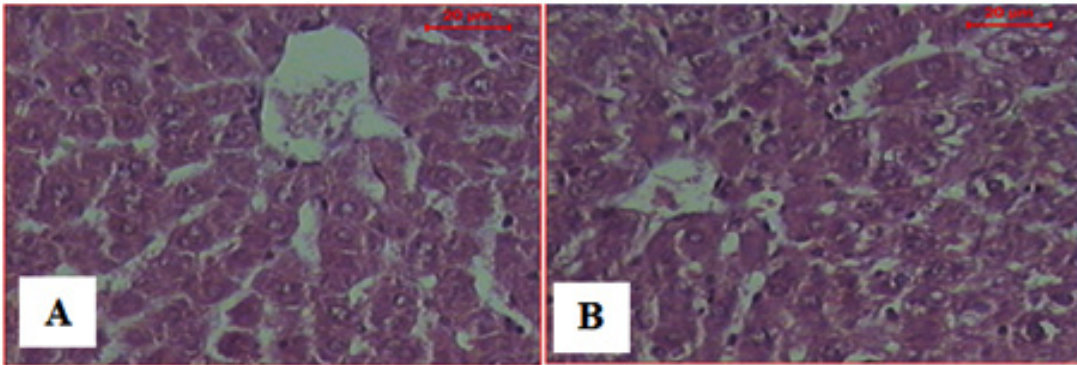


Fig. 4. Sections of liver of A) rat given paracetamol and Sum extract show the hepatic lobule that appear more or less like control, and B) rat given paracetamol and Sup extract show the hepatic lobule that appear more or less like control. Notice few foci of necrosis in the hepatocytes (H & E stain, Scale bar: 20 μ m)

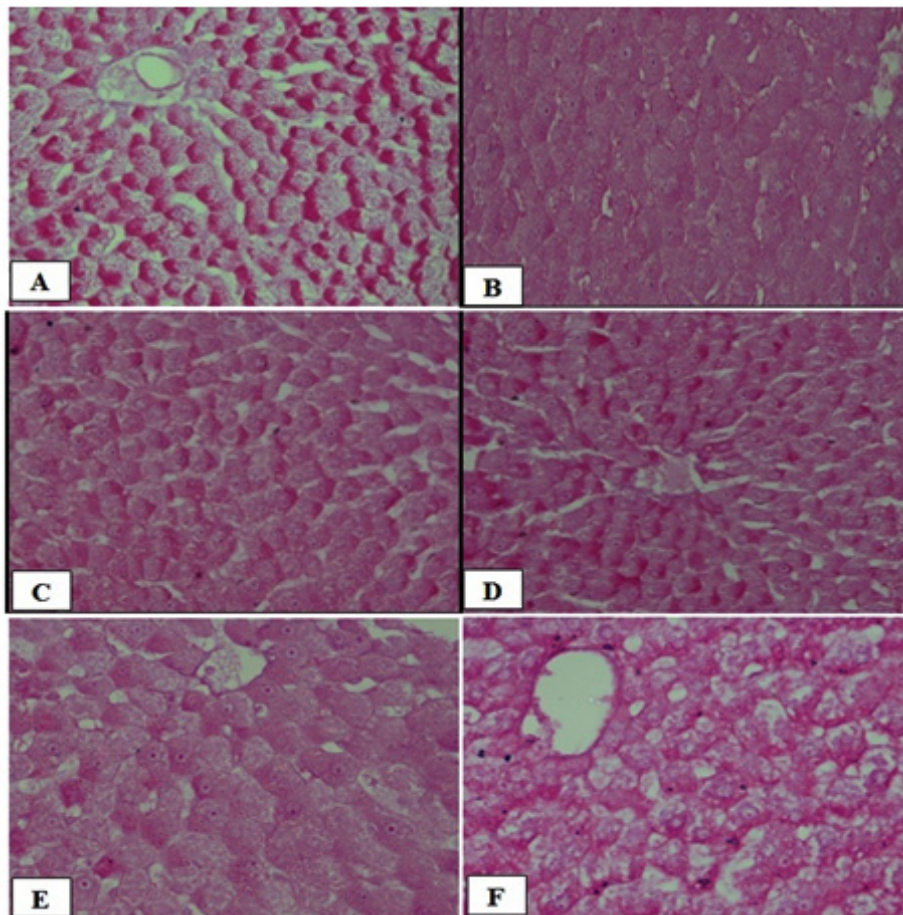


Fig. 5. Liver sections of A) normal rat showing normal distribution of glycogen in the hepatocytes, B) rat administered one dose of paracetamol (3 g/kg/b.w) showing a marked diminution in the glycogen contents as compared with control group, C, D): rats given Sum and Sup extracts for 7 consecutive days before a single dose paracetamol showing glycogen contents in the hepatic lobule appeared more or less like control, E, F) rats given Sum or Sup extracts for successive days before paracetamol showing heterogeneous distribution of glycogen. The healthy zones exhibit normal distribution and the injured zones exhibit a decrease in this inclusion (PAS stain, Scale bar: 20 μ m)

at a lower dose than N-acetylcysteine (NAC) in reducing APAP-induced hepatotoxicity; improving mouse survival rates, decreasing sALT and MDA accumulation and increase hepatic GSH synthesis in the mouse model⁴⁰.

Suaeda sp. may inhibit the activity of certain cytochrome P450 enzymes whose inhibition could safeguard against APAP hepatotoxicity³⁷. With regard to the potential hepatoprotective effects of *Suaeda*, it was demonstrated that rats which were chronically supplemented with Suaeda extract (100 mg/kg) and APAP (3 g/kg) exhibited an improvement in APAP-induced liver necrosis and increased serum ALT and AST levels⁵⁰. In the present study, paracetamol hepatotoxicity was associated with an increase in serum level of IL1 α . Similar results were reported by other investigators⁵¹⁻⁵².

Signaling through the IL-1 receptor (IL-1R) was recently shown to play an important role in paracetamol-induced hepatotoxicity⁵³. Activation of NF- κ B/Rel transcription factor leads to the increased production of various inflammatory mediators, including IL1 α and TNF- α .⁵⁴

Activation of Toll-like receptors (TLRs) by pathogen-associated molecular pattern results in upregulation of pro- IL1 α via NF- κ B pathway. This is followed by a second signal that results in a caspase 1 mediated cleavage of pro- IL1 α to release the active molecule⁵⁵.

Pretreatment with studied extracts decreased serum IL1 α and TNF- α in rats subjected to paracetamol hepatotoxicity. It was suggested that the two extracts have inhibitory effect on NF- κ B/Rel activity in rat serum⁵⁶.

In summary, our present study demonstrated that *S. monoica* and *S. peruisma* possessed a protective action against paracetamol-induced liver injury. The underlying mechanisms of their hepatoprotective activity might be attributed to the presence of the amino acids, unsaturated fatty acids and phenolic compounds. These encouraging findings would be helpful for developing potential hepatoprotective agents for remedying APAP-induced liver injury.

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