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Research Article

Biologically Active Polysaccharides from *Grewia asiatica* Linn. Leaves

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Abstract
The total ethanol, cold and hot polysaccharides (PS) of *Grewia asiatica* leaves were investigated for their *in vivo* hepatoprotective, antioxidant and anti-inflammatory activities. Carbohydrates content of leaves were investigated by paper chromatography and HPLC analysis which revealed the identification of eight sugars constituting 99.48% of free sugars fraction, six sugars in the cold and hot PS hydrolyzates constituting 88.47% and 92.24% respectively. The total ethanol extract, successive extracts, cold and hot PS were prepared and investigated for their hepatoprotective activity against carbon tetrachloride induced hepatotoxic rats. PS (cold and hot) exhibited a strong hepatoprotective and curative activities. The total ethanol and PS were screened for anti-inflammatory and *in vivo* antioxidant activities which revealed that both total ethanol and PS(cold and hot) exhibited good anti-inflammatory and antioxidant activities (52.77%, 69.13 , 82.45 and 96.58%,89.73%, 93.16% potency respectively) compared to indomethacin and vitamin E (100% potency).

Keywords: *Grewia asiatica*, hepatoprotective, anti-inflammatory, antioxidant, polysaccharides

INTRODUCTION

Grewia asiatica belongs to family Tiliaceae. It is commonly known as Phalsa, it is distributed throughout India. It is an important medicinal plant in Indian traditional system. Different parts of *Grewia* were used in folk medicine. The fruit of *G. asiatica* is astringent and cooling. Infusion of the bark is demulcent, febrifuge and used for treatment of diarrhea while leaves are used in postular eruptions. The root bark is used as a remedy for rheumatism. Ethanol extract of the aerial parts of *G. asiatica* showed a hypotensive activity while the aqueous extract of stem bark is reported to be antidiabetic[1]. The seed extract and seed oil exhibited antifertility activity[2]. Fruit Extract of *G. asiatica* shows radioprotective effect against γ-irradiation in Swiss Albino mice[3] The fruit is stomachic, anti-inflammatory and is administered in respiratory, cardiac and blood disorders, as well as in fever reduction[4]. The ethanol extract of *G. asiatica* showed *in vivo* hypoglycaemic activity[5]. Antidiabetic and antihyperlipidemic activities of the different extract of the leaves as well as the flavonoids and lipoidal contents were studied[6,7]. The Preliminary phytochemical study revealed presence of glycosides, fats, alkaloids, triterpenoids, sterols flavonoids, saponins, and tannins as active principals[5].

MATERIAL

Plant material

Fresh leaves of *Grewia asiatica* were obtained from the Zoo Garden, Giza, Egypt. The plant was authenticated by Therease Labib, Taxonomist of Orman Garden, Egypt. A voucher specimen was kept in the Herbarium of Pharmacognosy Department,, National Research Centre (NRC), Cairo, Egypt.

Authentic reference material for carbohydrates investigation

Glucose, galactose, rhamnose, xylose, arabinose, ribose, fructose, sucrose, glucuronic acid, galacturonic acid and sorbitol (Sigma)**.**

Reference materials for determination of total phenolic and flavonoids

Folin-ciocalteau reagent, gallic acid, querectin, $NaNO_2$, Na_2CO_3, $AlCl_3$

All previous chemicals were Merck products.

Experimental animals and diet

Animals

Table 1: Results of the quantitative determination of total carbohrdrates and total soluble sugars content of *G.asiatica* leaves.

Type	Percentage (g/100g dry wt)
Total carbohydrates	3.7
Total soluble sugars	2.5
% Polysaccharide (by difference)	1.2

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Table 2: Results of paper chromatographic investigation of free sugars fraction, cold and hot polysaccharide hydrolyzates of *G.asiatica* leav

Spot No.	R_f values		Free sugars	polysaccharide hydrolyzate		Colour with aniline phthalate	Authentic sugar
	S1 Solvent system system 2	S2		Cold	Hot		
1	0.590	0.693	+	+	++	Yellowish brown	Rhamnose
2	0.541	0.625	-	++	+	Brown	Ribose
3	0.383	0.564	--	--	--	Brown	Fucose
4	0.319	0.551	+	++	++	Redish brown	Xylose
5	0.271	0.517	--	--	--	Brown	Arabinose
6	0.254	0.481	__	--	--	Yellowish brown	Fructose
7	0.178	0.459	--	++	--	Brown	Glucose
8	0.162	0.447	+	+	++	Brown	Galactose
9	0.160	0.382	--	--	--	Pale brown	Glucuronic acid
10	0.156	0.297	++	--	++	Pale brown	Galacturonic acid
11	0.290	0.613	++	-	--	Brown	Mannose

S1- n-butanol- acetic acid – water (4: 1: 5 v/v) . S2-- isopropanol -water (4:1)
++ appreciably present ,+ present , - absent or not observed .

Table 3: Results of HPLC analysis of free sugars fraction, cold and hot polysaccharide hydrolyzates of *G.asiatica leave*

Authentic sugars	R_t	Relative %		
			Polysaccharide hydrolyzate	
		Free sugars	Cold	Hot
Fucose	1: 08	--	--	--
Rhamnose	1:41	4.17	9.03	18.58
Maltose	1:76	-	-	--
Lactose	1:80	16.15	--	--
Sorbose	1:93	9.08	--	4.01
Galactose	2:21	4.78	4.27	10.98
Galacturonic acid	2:40	9.86	--	11.26
Ribose	2:62	-	16.75	8.34
Xylose	2:89	3.88	13.78	39.07
Sorbitol	3:19	0.28	--	-
Arabinose	3:39	-	--	-
Fructose	3:88	-	--	-
Mannose	4:29	51.28	1.13	-
Glucose	4:46	-	43.51	-
Glucuronic acid	5:50	--	--	-
Sucrose	6:41	--	--	
unknowns		0.52	11.53	7.76
Total identified sugars		99.48%	88.47	92.24
				-

Adult albino rats, of Sprauge Dawely strain weighing 130-150 g and, Albino mice weighing 25-30g were obtained from the animal house colony of NRC, Dokki, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water. Medical Research Ethical Committee (MREC) in NRC has approved the work.

Normal diet

It consisted of vitamin mixture (1%), mineral mixture (4%), corn oil (10%), sucrose (20%), cellulose (0.2%), casein 95% (10.5%) and starch (54.3%).

Doses

Doses of the drugs were calculated and administered orally by gastric tube

Material for biological studies

- Carbon tetrachloride (Analar) is used as a hepatotoxic agent.
- Biodiagnostic kits are used for the assessment of blood glucose, serum AST, ALT, ALP and blood glutathione levels (QCA, Spain).
- Silymarin (SEDICO, Cairo, A.R.E) is used as a standard hepatoprotective drug.
- Alloxan (Sigma Co) was used for the induction of diabetes in rats

Table 4: Effect of *G.asiatica* leaves ethanol extract, cold and hot polysaccharides and silymarin drug on serum AST enzyme level in liver damaged rats

Group	AST (U/L)			
	Zero	7d	72h	7d
Control 1 ml Saline	42.4 ± 2.4	41.2± 1.4	153.6 ± 4.9*	166.9 ±5.8*
Total extract	46.3± 1.9	45.9 ± 1.5	83.1 ± 3.8*	56.2 ± 2.9•*
Petroleum ether	43.7 ± 1.2	42.1± 1.8	98.4 ± 3.6*	91.4 ± 2.5 *
Chloroform	39.8 ± 1.4	40.2 ± 1.6	106.2 ± 3.8*	89.7± 2.4•*
Ethyl acetate	41.4 ± 1.3	41.1± 1.3	86.9 ± 2.8*	71.2± 2.3•*
Aqueus ethanol	40.9± 1.7	39.3±1.4	79.8±2.6*	67.8 ±2.8*
Cold Polysaharides	38.4±1.6	37.5 ±1.7	71.6 ±2.9*	53.8±2.2•*
Hot Polysaharides	36.3± 1.2	35.7 ±1.3	65.2 ±2.4*	46.1 ±2.3•*
Silymarin 25mg/kg	44.3± 1.6	42.3± 1.7	61.2 ± 2.1*	42.7 ± 2.6•

Table 5: Effect of *G.asiatica* leaves ethanol extract cold and hot polysaccharides and silymarin drug on serum ALT enzyme level in liver damaged rats

Group	ALT (U/L)			
	Zero	7d	72h	7d
Control 1 ml saline	38.7 ± 1.3	38.1± 1.6	149.2 ± 6.1*	156.4 ±5.9*
Total ethanol	36.4 ± 1.2	41.9± 1.8	74.5 ± 2.4*	49.7± 1.3•*
Petroleum ether	41.1. ± 1.4	38.8± 1.4	112.4± 2.6*	91.6 ± 3.2•*
Chloroform	40.1 ± 1.5	42.7± 1.2	103.7± 4.3*	89.7± 3.1•*
Ethyl acetate	43.2 ± 1.3	43.2 ± 1.6	78.7± 2.9*	65.9 ± 3.2*
Aqueus ethanol	41.5± 1.4	39.3±1.4	83.2±2.7*	52.8±2.1•*
Cold Polysaharides	33.4 ±1.2	31.1± 0.8	78.1 ±2.6*	47.4± 2.1•*
Hot Polysaharides	31.2± 1.1	30.5 ±0.9	61.8 ±2.1*	39.2± 1.3•*
Silymarin	39.6± 1.8	38.6 ± 1.3	49.8± 1.7*	38.25 ± 1.4•

Table 6: Effect of *G.asiatica* leaves ethanol extract cold and hot polysaccharides and silymarin drug on serum ALP enzyme level in liver damaged rats.

Group	ALP (KAU)			
	Zero	7d	72h	7d
Control 1 ml Saline	7.3± 0.1	7.2 ± 0.1	49.2± 1.8*	58.7±7.1•
Total ethanol	7.1± 0.1	6.9 ± 0.1	21.1 ± 0.7*	17.9± 0.4*
Petroleum ether	7.5± 0.1	7.4 ± 0.1	33.6 ± 1.1*	28.7 ± 0.9•*
Chloroform	7.2 ± 0.1	7.1 ± 0.1	36.7 ± 1.4*	32.4± 1.1•*
Ethyl acetate	7.6± 0.1	7.3 ± 0.1	26.2 ± 0.9*	22.9± 0.4•*
Aqueus ethanol	7.4± 0.1	7.2±0.1	24.6±0.6*	23.2 ±0.3*
Cold Polysaharides	7.6± 0.1	7.5 ±0.1	36.4± 1.9*	23.4 ±0.6•*
Hot Polysaharides	7.2 ±0.1	7.1 ±0.1	31.2±1.8*	18.1± 0.3•*
Silymarin	7.6± 0.1	7.5 ± 0.1	16.2± 0.6*	7.3± 0.1•

* Significantly different from zero time at $p<0.01$.

• Significantly different from 72hr after CCl_4 at $p<0.01$

- Vitamin E (dl *α*-tocopheryl acetate) (Pharco Pharmaceutical Co). is used as a reference antioxidant
- drug. It is available in the form of gelatinous capsules; each contains 400 mg vitamin E.
- Carrageenan (Sigma Co.) was used for the induction of
- acute inflammation in rats
- Indomethacin (Kahera Pharm. Ind. Co. A.R.E.) was used as a standard anti-inflammatory drug

METHODS

Phytochemical study

Quantitative determination of carbohydrates

Determination of the total carbohydrates content

Total carbohydrates were determined as glucose by phenol-sulphuric acid method[8] as follows: 5 g of the vacuum oven-dried powdered plant (at 45^0C) were mixed with 10 ml 1M sulphuric acid and refluxed on a boiling water bath for two hours. The hydrolyzed sample filtered off and the volume was adjusted to 100 ml in a volumetric flask with distilled water. To 0.5 ml of the filtrate, 0.5 ml of 5% phenol solution was added, followed by 2.5 ml conc. sulphuric acid. The mixture was allowed to stand for 10 minutes, in a water bath at 25°C to 30 °C. The absorbance of the characteristic yellow-orange color was measured at 490 nm, against a blank. The percentages of total carbohydrates in the sample was determined by reference to the standard curve of glucose applying the following equation:

% of sugar =y/m × 100/weight of the sample.

Where *y* = mean reading, *m* = slope (0.003)

Determination of the free sugars content[9]

Table 7: Acute anti-inflammatory activity of the total ethanol extract cold and hot polysaccharides of *Grewia asiatica* Linn. leaves

Time (hour)	Zero oral dose of extracts	1hr after oral dose of extract Carragenan injection		2hrs		3hrs		4hrs		% potency after 4hr
	Paw diameter (mm) Mean ± S.E	Paw diameter Mean ± S.E	% Oedema	Paw diameter Mean ± S.E	% Oedema	Paw diameter Mean ± S.E	% Oedema	Paw diameter Mean ± S.E	% Oedema	
Control	3.38±0.09	4.41 ±0.1*	30.47	4.81 ±0.13*	42.30	4.89± 0.12*	44.67	4.96 ±0.08*	46.74	-
Total ethanol	3.54±0.06	4.26±* 0.05	20.33	4.05 ±0.01*	14.40	3.93 ±0.09*	11.01	3.95± 0.2*	11.58	52.77
Cold polysa	3.51±0.01	4.39±0. 07*	25.07	4.25 ±0.08*	21.08	4.15 ±0.05*	18.23	3.96± 0.01*	12.82	69.13
Hot polysa	3.47±0.9	4.48±0. 06*	29.11	4.27 ±0.5*	22.19	4.06 ±0.01*	17.00	3.95± 0.2*	13.83	82.45
Indomethacin	3.56±0.08	4.26±0. 09*	19.66	3.99 ±0.06*	12.08	3.92 ±0.01*	10.11	3.83 ±0.01*	7.58	100

* Significantly different from zero time at $p<0.01$

5g of the vacuum oven-dried powdered plant (at 45°C) were extracted under reflux with 20 ml ethanol (80 %) on a water bath (at 70°C) for one hour. After removal of ethanol, the volume was adjusted with distilled water to 100 m1 in a volumetric flask. The procedure was completed as mentioned above under the determination of total carbohydrates.

Qualiative determination of carbohydrates

Investigation of free sugars content

50 g of defatted powder of leaves of *G.asiatica* L were extracted with 80% ethanol for 24 hrs. The extract was evaporated and the residue was kept for chromatographic investigation and HPLC analysis.

Investigation of polysaccharides content.

The marc remained after complete extraction with 80% ethanol were successively percolated with cold followed by hot water till complete exhaustion in each case[10] (till -ve -Molisch's test[11]). Cold and hot aqueous extracts of each powdered plants were separately concentrated to 100 ml. Absolute ethanol (300ml 3x) was added drop wise till complete precipitation occurred, then precipitated polysaccharides were collected by centrifugation. The precipitate obtained in each case was washed several times with absolute ethanol then stirred with acetone, filtered, dried, in vacuum desiccators and weighed (Table 1). Part of the cold and hot polysaccharides were kept for testing the biological activities.

Acid hydrolysis of polysaccharides

100 mg of each of cold and hot polysaccharide extracts of leaves of *G.asiatica* were heated in 2 ml of 0.5 M sulphuric acid in sealed tubes for 20 hrs in a boiling water bath [12]. At the end of hydrolysis, a flocculent precipitate was noticed. This was filtered off and the filtrate was freed of SO_4 by precipitation with barium carbonate. The solution was evaporated to dryness and the residue was kept for chromatographic investigation and HPLC analysis.

Paper chromatographic investigation

A part of the free sugar fraction, cold and hot hydrolyzate residues as well as authentic sugars were dissolved in 10% isopropanol/ H_2O then spotted on Whatmann1MM. The chromatograms were developed adopting the descending technique for 18 hrs, using the two solvent systems *n*- butanol - acetic acid – water (BAW 4:1:5 v/v, upper layer), isopropanol water (4:1) [13]. The chromatograms were visualized by spraying with aniline phthalate reagent and heating in an oven at 110 °C for 5 min. The results are presented in Table 2

HPLC analysis

10 mg of free sugars fraction, cold and hot hydrolyzate residues as well as of the individual authentic sugars were separately homogenized with acetonitrile/H_2O (76/24 v/v). The extracts were filtered through a Whatman no.1 micro filter (0.45 μm) and partitioned three times with ethyl acetate and stored in vials. The HPLC analysis was used to determine sugars in the extracts. The analysis was performed on a model HP1050 HPLC. Separation and determination were performed on APS column (4.6 × 200 mm). The mobile phase was the same used in the extraction, flow rate (2 ml. min-1)[14]. The results are listed in Table 3.

Quantitative determination of total phenolic acids and flavonoids

Extraction

Total phenolic acids and total flavonoids assay was conducted using the modified method of Marinova et al.[15]. 0.5gm of powdered *G.asiatica* leaves was extracted using 50 mL of 80% aqueous methanol on an ultrasonic bath for 20min. Then the mixture was centrifuged for 5 min at 14,000 rpm. The supernatant was collected and used for total phenolic acids and total flavonoids quantification.

Table 8: Antioxidant activity of total ethanol extract cold and hot polysaccharides of *Grewia asiatica* Linn. leaves

Group (dose in mg /kg.b.w)	Blood Glutathione (Mean±SE)	% of change from diabetic	Relative potancy%
-ve (control)	36.7 ±1.4	-	-
Diabetic +ve (control)	21.8±0.5	-	-
Diabetic + total ethanol (100 mg/kg)	35.9±1.2*	64.68	96.58
Diabetic +cold PS(100 mg/kg)	34.9±0.9*	60.09	89.73
Diabetic +hot PS(100 mg/kg)	35.4±1.1*	62.39	93.16
Diabetic +vit E(7.5mg/kg)	36.4±1.3*	66.97	100

Significantly different from diabetic control at $p<0.01$

Determination of total phenolics

Total phenolic (TP) compounds were assayed using the Folin-Ciocalteu assay[15]. 1 mL extract or standard solution of gallic acid (20, 40, 60, 80 ,100mg/l) was added to a flask containing 9 mL distilled water. Then 1 mL of Folin-Ciocalteu's phenol reagent was added and the mixture was mixed and shaken. After 5 min 10 mL of 7% sodium carbonate was added, the mixture was then completed to 25 mL with distilled water mixed and allowed to stand at room temperature for 90 min. The absorbance against the prepared reagent blank was determined at 750 nm. using a spectrophotometer (UV–VIS, Labomed Inc USA). TP content was expressed as mg gallic acid equivalents (GAE)/g samples

Determination of total flavonoid

Total flavonoid (TF) was determined using the aluminum chloride colorimetric method[15]**.**

1 mL of plant extract or standard solution of querectin (20, 40, 60, 80, 100mg/l) was added to 4 mL distilled water in a 10 mL flask. 0.3 mL of 5% $NaNO_2$ was added after 5 min 0.3 mL of 10% $AlCl_3$ was added and left for 6 min then 2 mL of 1 M NaOH was added. The mixture was diluted to 10 mL with distilled water. The absorbance of the solution was measured at 510 nm using a spectrophotometer (UV–VIS, Labomed Inc USA.). The results were expressed as mg querecetin equivalents (Qu)/g samples all samples were analyzed in duplicate

Biological study

Preparation of extracts

Total extract

Five hundred g of air dried powdered leaves of *G.asiatica* were exhaustively extracted by reflux with 80% ethanol. The extract was evaporated under reduced pressure.

Successive extracts

Five hundred gm. of air dried powdered leaves of *G.asiatica* were exhaustively and successively extracted in a Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and aqueous ethanol(70% ethanol). These extracts were evaporated to dryness under vacuum at 40°C they were kept for biological investigation**.**

Determination of hepatoprotective activity

Liver damage in rats was induced according to the method of Klassen and Plaa**[16]** by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride in liquid paraffin. Forty four male albino rats were divided into nine groups each of six rats as follows:

First group: control group received a daily oral dose of 1 ml saline for 7 days before and after liver damage (negative control).

Second to eighth group: liver damaged rats pretreated with daily oral dose of 100 mg/kg b.wt. of total ethanol extract, petroleum ether, chloroform, ethyl acetate, aqueous ethanol extracts, cold and hot polysaccharide respectively for 7 days.

Ninth group: liver damaged rats pretreated with daily oral dose of 25 mg/kg b.wt. silymarin as a standard. Administration of the extracts and drug was continued after liver damage for another 7 days, followed by overnight fast, whole blood was obtained from the retro orbital venous plexus through the eye canthus of anesthetized rats. Blood samples were collected at zero time, 7 days before CCl_4 injection, 72 hours and 7 days after CCl_4 injection. Serum was isolated by centrifugation. Serum AST, ALT[17], and ALP[18] were measured. Results are expressed as mean ± S.E. The data was statistically analyzed using the Student's "t" test[19]. The results are considered statistically significant with $P > 0.01$. The results are compiled in Tables 4- 6

Determination of acute anti-inflammatory activity

Paw swelling, is a convenient method for assessing inflammatory responses to antigenic challenges and irritants This effect was determined according to the method described by Winter *et al*.[20] This model uses carrageenan (C) as the irritant to induce paw oedema. Typically, test materials are assessed for acute anti-inflammatory activity by examining their ability to reduce or prevent the development of carrageenan-induced paw swelling.

Thirty male albino rats weighting 130-150g were divided into five groups, each of six animals, first group received 1ml of saline serving as control, second to forth group received 100 mg/kg of total ethanol, cold and hot polysaccharides of leaves fifth group received 20 mg/kg of the reference drug indomethacin. One hour after oral administration of extracts, all animals were given a sub-plantar injection of 0.1 ml of 1 % carrageenan solution in saline in the right hind paw and 0.1 ml saline in the left hind paw. The oedema diameter was measured by the caliber at 1, 2, 3, 4 hrs after extract administration and % oedema was calculated and results are listed in Table 7

$$\% \text{ Oedema} = \frac{(PdaC - PdbC)}{PdbC} \times 100$$

Where, Pda: paw diameter after

Pdb: paw diameter before

Determination of antioxidant activity

Glutathione (GSH) is the body's natural antioxidant. A lack of GSH has been shown to leave the body more vulnerable to damage by free radicals, thus speeding up oxidations in the body. The diabetogenic process appears to be caused by immune destruction of the beta cells; part of this process is apparently mediated by production of active oxygen species. Diabetes can be produced in animals by the drugs such as alloxan which result in the production of active oxygen species[21]. So there was a fall in blood GSH was observed following the injection of diabetogenic doses of alloxan. Therefore, blood glutathione was estimated in alloxan induced diabetic for studying the antioxidant activity. Thirty six male albino rats were divided into six groups, each of six animals, as follows: First group: received 1 ml saline and kept as a negative control. Diabetes was induced in the other groups, according to the method described by Eliasson and Samet[22], using a single dose of intraperitoneal injection of 150 mg/kg b.wt. alloxan, followed by an overnight fast. Second group: diabetic rats that kept untreated (positive control). Third, fourth and fifth diabetic rats that received a dose of 100 mg/kg b.wt. of total ethanol extract, cold and hot polysaccharides of leaves. Sixth group diabetic rats that received 7.5 mg/kg of vitamin E as a reference drug. The rats received the extracts and the standard drug for seven days, at the end of the experiment, blood glutathione was estimated using biodiagnostic kits[23] (Table, 8)

RESULTS AND DISCUSSION

Carbohydrate Investigation

Polysaccharides, are complex homo or hetero polymers of monosaccharides joined together by glycosidic linkage. The isolated carbohydrates were pale grey in color, odorless, tasteless, and soluble in water, insoluble in ethanol, ether and chloroform. They gave positive Molische's test[11]. The percentage of total carbohydrates as determined by phenol sulphuric acid is 3.7% while that of free sugars was 2.5%. HPLC analysis of low Mwt. carbohydrates contents (free sugars) revealed the identification of eight sugars in 80% ethanol extract constituting 99.48% of the total fraction; the major sugar was mannose (51.28%) lactose (16.15%), galacturonic acid (9.86%) and sorbose (9.08%). Six sugars in the cold and hot polysaccharide hydrolyzate were identified constituting 88.47% and 92.24% respectively of the total fraction. Glucose (43.51 %), ribose (16.75 %) xylose (13.78 %) and rhamnose(9.03%) were the major sugars of cold hydrolyzate while xylose(39.07%) rhamnose (18.58%) galacturonic acid (11.26%) galactose(10.98%) were the major sugars of hot hydrolyzate The total phenolic and total flavonoid contents in the total ethanol extract were found to be 165 mg eq GA/ g extract, 87 mg eq Qu/ g extract respectively

Biological study

CCl_4 is a hepatotoxic agent commonly used for the production of liver toxicity in experimental models[24]. The hepatotoxic effects of CCl_4 are largely due to generation of free radicals[25]. CCl_4 is biotransformed by the cytochrome P_450 system to trichloromethyl free radical ($\bullet CCl_3$) which reacts with oxygen to form trichloromethyl-peroxy radical ($\bullet CCl_3O_2$), this reactive metabolite attack polyunsaturated fatty acids of the cell membrane causing lipid peroxidation leading to impairment of membrane function and liver injury[26]. Damage of liver cell led to increase in the levels of hepatospecific enzymes which are cytoplasmic and as a result they released into circulation after cellular damage[27]. The serum transaminases level is most widely used as a measure of hepatic injury, due to its ease of measurement and high degree of sensitivity. It is also useful for the detection of early damage of hepatic tissue and requires less effort than that required for a histological analysis.

In this study there is a significant increase in the levels of AST, ALT & ALP in the toxicant group. Pretreated groups with different plant extracts showed reduction in biochemical parameters AST, ALT & ALP in addition to protective activities to groups which receive total ethanol, polysaccharides, ethyl acetate and aqueous ethanol extracts these extracts have protective and curative activity in liver damaged rats as compared with silymarin.(Tables 4,5&6) Polysaccharide isolated from the plant (cold and hot) exhibit a strong hepatoprotective and curative activities, in the other hand hot fraction containing galacturonic acid has the highest significant protective and curative activity on the hepatotoxicity of CCl_4, indicating that the presence of uronic acids in the polysaccharide complex may increase the hepatoprotective effect[28].

The hepatoprotective activities of ethyl acetate and aqueous ethanol extract may be due to presence of flavonoids which are previously isolated[6], moreover total ethanol extract possess proteins and mucilage which also are reported to have hepatoprotective activity[29].

The significant hepatoprotective activities of the total ethanol and hot PS, cold PS to hepatotoxic rats, encourage us to study the possible anti-inflammatory and antioxidant properties.

Carrageenan-induced edema, involves the release of mediators at the injured site, these mediators include prostaglandins, especially the E series, histamine, bradykinins, leukotrienes, and serotonin. These mediators cause pain and fever, swelling[30]. Carrageenan-induced rat paw is a suitable experimental animal model for evaluating the anti-edematous effect of natural products and is a significant predictive test for anti-inflammatory agents acting on the mediators of acute inflammation[31]. Significant anti-inflammatory activities were exhibited by oral administration of doses of different extracts. All extract inhibited the rat paw edema weight induced by carrageenan, this effect was found to be time dependant the most potent extract was the total ethanol(52.77%) followed by cold PS(69.13%) and hot PS(82.45 %) comparable to indomethacin(100%).(Table 7). It should be noted that the anti-inflammatory activities of many plants have been attributed to their triterpene[32] or flavonoid contents[33,34]. It has been also demonstrated that various flavonoids (such as rutin, quercetin, and luteolin),

biflavonoids, and triterpenoids (such as ursolic acid) produced significant antinociceptive and/or anti-inflammatory activities[35],[36]. *G.ascitica* were previously studied for their flavonoids and terpenoids compounds[6,7], therefore, the anti-inflammatory activity of the total ethanol extract may be due to flavonoids and terpenoid contents. Non-steroidal anti-inflammatory drugs (NSAID) such as indomethacin act by the reduction of sensitization of pain receptors caused by prostaglandins at the inflammation site[37]. The observed anti-inflammatory activities of these extracts may be attributed to the overall effects of the plant constituents or the compounds having actions similar to NSAID. In conclusion, it can be expected that crude plant material with active flavonoids, phenolic acids, PS and terpenoids contents will be effective in therapy.

Glutathione (GSH) is natural antioxidant in the animal body. A lack of GSH expose the body to damage by free radicals, thus speeding up oxidations in the body. The diabetogenic process appears to be caused by immune destruction of the beta cells, part of this process is mediated by production of active oxygen species. Alloxan was used to induce diabetes in animals which result in the production of active oxygen species[21]. A simultaneous fall in blood GSH was observed following the injection of diabetogenic doses of alloxan into rabbits[38]. The antioxidant activity of the total ethanol may be due to presence of flavonoids which are powerful free radical scavenging activity[39] they exert there action through a membrane stabilizing action and inhibiting lipid peroxidation of cell membrane[40]. *In vivo* antioxidant activity was evaluated as indicated by the increase in glutathione level as compared with diabetic control, the result showed that the ethanol extract exhibited the highest free radical scavenging activity (96.58%potency) followed by the HPS (93.16%) and CPS (89.73% potency) as compared with the standard antioxidant vitamin E (100% potency), Table 8). The present study about the biological activities of polysaccharides were matched with the reported results about the different biological activities of polysaccharides as antioxidant[41], hepatoprotective[42],[43] and anti-inflammatory[44]

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