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COMPARATIVE ANTI-INFLAMMATORY AND HEPATOPROTECTIVE ACTIVITIES OF *ASTRAGALUS GUMMIFER* LABILL HERB AND ROOTS IN RATS

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Abstract

Background: The *Astragalus gummifer* (F. Fabaceae), herb and roots were studied for anti-inflammatory and hepatoprotective activities.
Materials and method: The alcoholic extracts of *Astragalus gummifer* (F. Fabaceae), herb (AGHE), and roots (AGRE), were used for anti-inflammatory and hepatoprotective activities in Wister rats. The effects of AGHE and AGRE were compared with the standard drugs Phenylbutazone and silymarin, for anti-inflammatory and hepatoprotective activities respectively.
Result: Both extracts showed significant anti-inflammatory activity ($P< 0.001$). AGRE showed comparatively more significant hepatoprotective activity ($P< 0.001$), than AGHE ($P< 0.05$); at doses of 250 and 500 mg/kg body weight as manifested by lowering the serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), and total bilirubin. The hepatoprotective activity was, also, supported by total protein (TP), malondialdehyde (MDA), nonprotein sulfhydryls (NP-SH), and histo-pathological studies of liver tissue.
Discussion: To the best of our knowledge, this is the first report of the anti-inflammatory and hepatoprotective activities of *Astragalus gummifer*. The results of present studies indicated that both AGHE and AGRE can be used in inflammatory conditions, while investigation supports the use of AGRE in cases that hepatoprotection are required in the hepatotoxic conditions. More supportive studies are required before clinical recommendation.

Key Words: *Astragalus gummifer*, Anti-inflammatory, Hepatoprotective, CCl4, carrageenan, Biochemical

Introduction

Astragalus gummifer Labill (Fabaceae) is distributed broadly throughout the world, and predominantly in Europe, Asia and North America. *Astragalus* species is represented by approximately 3000 taxa in the world. In Turkey, it is represented by 463 taxa (including subspecies and varieties), 210 (41%), of them are endemic (Muhittin et al., 2013). Roots of *Astragalus* species are used to treat leukemia and for wound healing in Turkish folk medicine (Yesilada et al., 2005). A wide range of pharmacological properties have been reported for *Astragalus spp* including immune-stimulant, anti-bacterial, antiviral, hepatoprotective, anti-inflammatory, cardiovascular tonic, and vasodilatory action, and in treatment of diabetes mellitus, nephritis, leukemia and uterine cancer (Bedir et al., 2000; Gariboldi et al., 1995; Rios and Waterman, 1997; Pistelli et al., 2002). The two major biologically active constituents, reported in *Astragalus* species, are polysaccharides and cycloartane-type saponins (Tang and Eisenbrand, 1992; Lee et al., 2013). About 70 cycloartane-type saponins including five different aglycones have been investigated from the Turkish *Astragalus* species (Linnek et al., 2011; Polat et al., 2010; Horo et al., 2010; Sevimli-Gür et al., 2011).

The mechanism of the effectiveness of Astragalus is still poorly understood (Auyeung et al., 2009; Yejin and Yanqun, 2010). The effect of Astragalus polysaccharides (APS), on the immune system has been studied extensively; however, there are few published reports about the Astragalus saponin (AS), induced immune responses. Macrophyllosaponin B (Mac B, the major saponin of the most active species *Astragalus oleifolius*), and Astragaloside VII (AST VII, the most active compound), possess prominent IL-2 inducing activity and might have a contributory role in the immuno-stimulating and anticancer effects of *Astragalus* species with anti-inflammatory activity (Nalbantsoy et al., 2011). Chronic diseases connected with persistent inflammatory stimuli are commonly associated with an increased risk of cancer development (Coussens and Werb, 2002; Philip et al., 2004). The appropriate medications are used principally to treat inflammation. Recently it has been reported that Astragalus saponins (AS), stimulate apoptosis and Astragalus polysaccharides (APS), stimulate macrophages to express iNOS gene (Lee and Y Jeon, 2005). A saponin isolated from the *A. membranaceus* was reported as an antioxidant and antidiabetics agent (Adiguzel et al., 2009).

The antioxidant action of another sub-species, *A. mongholicus*, was also investigated (Yu et al., 2005). Flavonoids are found in most parts of the plant species and are responsible for the multiple biological activities such as anticarcinogenic, anti-inflammatory, antibacterial, antiviral and immune-stimulating and improve the atherosclerosis effects (Wang et al., 2012). Treatments for common liver diseases such as cirrhosis, fatty liver and chronic hepatitis are problematic. The effectiveness of treatments, such as interferon, colchicines, penicillamine and corticosteroids are inconsistent at best and the incidence of side-effects profound (Strader et al., 2004). Several studies have demonstrated the protective effects of antioxidants against induced liver injury by reducing oxidative stress in cells (Cederbaum et al., 2009). A number of phytochemicals showing promising activity, including Silymarin, are considered as reference drug and widely used for the treatment of hepatitis and liver cirrhosis (Dvorák et al., 2003).

In the present study, we aimed to report the anti-inflammatory and hepatoprotective activity of *Astragalus gummifer* herb and roots.

Materials and Methods

Materials

Carrageenans (BDH), Phenylbutazone (PBZ), Silymarin, Lipid Per oxidation (MDA), Assay Kit, EDTA, Trichloroacetic acid (TCA), DNTB were purchased from Sigma Aldrich.

Collection and Authentication of plant

Astragalus gummifer Labill (Fabaceae) was collected from Elazig-Hazargolu road, 23 km southeast of Elazig, East Anatolia, Turkey, in June 2000. A voucher specimen has been deposited in the Herbarium of the Pharmaceutical Botany Department, Faculty of Pharmacy, Hacettepe University, Ankara Turkey (HUEF 00-23). The plant has been authenticated by Prof. Dr. Zeki Aytac Gazi University, Department of Biology, Faculty of Science and Art, Ankara, Turkey.

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Extraction

Collected herb and roots were separately chopped into small pieces, dried in shade, and then soaked in 80% ethanol for 72 hrs. The extract was filtered and the filtrate was subjected to rotary evaporation (50°C). The thick solution of *Astragalus gummifer* herb extract (AGHE), and root extract (AGRE), were lyophilized using freeze drier and the obtained extract 16 and 20 g respectively were stored in a freezer at -80°C and used for the various experimental studies.

Animal model:

Both sexes of adult albino mice (25–30 g b. wt), were used in the acute toxicity test. Wistar albino rats (200-250g), of either sex were used in anti-inflammatory and hepatoprotective studies. Animals were obtained from Lab Animal Care Unit, Pharmacy College, Salman bin Abdulaziz University, Al-Kharj, KSA. All animals were kept under uniform and controlled conditions of temperature and light/dark (12/12 h), cycles, fed with standard rodent diet and given fresh purified potable water *ad libitum*. The animals were allowed to acclimatize to the laboratory condition for one week before the commencement of the experiment. The experimental procedures used were approved by the Ethical Committee of the College of Pharmacy, King Saud University, Riyadh.

Determination of acute toxicity and median lethal dose (LD_{50}) of the extracts

LD_{50} of AGHE and AGRE were determined according to the reported method (Tanko et al., 2008). Mice were divided into groups of 6, and the tested extracts were administered orally in doses of 100 to 3000 mg/kg body weight. Signs of acute toxicity and number of death per dose within 24 hrs were recorded and the LD_{50} was calculated.

Carrageenan-induced paw edema in rats

Pedal inflammation in albino rats of either sex 200-250g was produced according to the previous method (Winter et al., 1962). Injection of 0.05ml of 1% carrageenan sodium salt was given through the right hind foot of each rat under the plantar aponeurosis. The test groups of rats were treated orally using 250 and 500mg/kg AGHE and AGRE, separately, one hour before carrageenan injection. At the same time, control group was given 5 ml/kg of normal saline and the reference group was given 100mg/kg of an aqueous solution of phenylbutazone. The measurement of foot volume was done by the displacement technique using Plethysmometer (Apelex, France), immediately after 2 to 3 hrs of administering the carrageenan injection. The inhibitory activity was calculated according to the following formula

$$100\frac{1-a-x}{b-y}$$

Where "b" is the mean volume of control rats after carrageenan injection and "y" before the carrageenan injection; "x" is the mean paw volume of treated rats before injection and "a" is the mean paw volume after carrageenan injection

Hepatoprotective study

The Hepatoprotective activity was evaluated in Wister albino rats using CCl_4 induced liver injury (Mistry et al., 2013). The rats were divided into seven groups (n=5); Group-1 served as control (normal saline), Group II served as hepatotoxic (CCl_4), Group III, served as positive control (Silymarin). Group IV & V served as (250 and 500 mg/kg b.w.) AGHE treated groups, while Group VI & VII served as (250 and 500 mg/kg b.w.), AGRE treated groups. Animals were sacrificed, under light ether anesthesia, 24-h after the last dose. Blood was collected by cardiac puncture in plain tubes and liver was removed, rinsed in cold saline, blotted with filter paper and weighted. Serum was separated by centrifugation at 3000 rpm at 4°C for 10 min. 10% (w/v), liver homogenate was prepared in 0.25M sucrose solution and centrifuged at 7000 rpm for 10 min at 4ºC and the supernatant was used for various biochemical assays. The ventral portion/s of the left lateral liver lobe were collected and fixed in 10% neutral-buffered formalin for histopathological analysis.

Biochemical Assays

The measurement of various biochemical parameters such as serum ALT, AST, ALP, GGT and total bilirubin were estimated using isolated serum according to the reported methods (Poojari et al., 2009). The enzyme activities were measured using diagnostic strips (Reflotron ®, ROCHE), and were read on a Reflotron® Plus instrument (ROCHE). Protein concentration was estimated according to the method (Lowry et al., 1951) using bovine serum albumin (BSA) as a standard.

Determination of Malondialdehyde (MDA)

The measurement of MDA has been used as an indicator of lipid per oxidation (Alqasoumi, 2010), and assayed by Satoh method. In brief, 10% (W/V) liver homogenate in 0.1 M/L phosphate buffer was centrifuged at 4°C, 3500 rpm for 10 min. 2 ml supernatant was mixed with 67% 2-thiobarbituric acid and 20% trichloroacetic acid solution then heated in water bath at 95°C for 5 min. The latter tube was centrifuged and the supernatant was collected. The pink color chromogen, formed by the TBA with MDA, was measured at 532nm. The result was expressed as MDA nmol/mg protein.

Estimation of Non-Protein sulfhydryls (NP-SH)

The NP-SH was measured according to the reported method (Sedlak and Lindsay, 1968). The tissue was homogenized with ice-cold 0.02 mmol/l ethylene diamine tetra acetic acid (EDTA). Aliquots of 5 ml of the homogenate were mixed with 4 ml of distilled water and 1 ml of 50% TCA in 15ml test tubes. The homogenate was centrifuged at 3000g. 0.1 ml of the supernatant was suspended in tris buffer, 5-5'-dithiobis-(2 nitrobenzoic acid) (DTNB), and observance was measured within 5min at 412 nm against reagent blank with no homogenate**.**

Histopathological studies

A small fragment of liver tissues was placed in 10% formalin (diluted to 10% with normal saline), for 1 hr to rectify shrinkage due to

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high concentration of formalin according to the previous method (Alqasoumi, 2010). The tissues were dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight and 100% isopropyl alcohol for 1 hour and finally paraffin wax (four times 1 h). Tissues were transferred in to paraffin waxed filled moulds. The rotary microtome (Leitz 1512), was used for making the section (3 µm). The sections were placed on clean slides and place onto warming table at 37-40^{0}C. The slides were then stained for 15 min with Mayer's hematoxylin solution, washed for 15 minutes in lukewarm running tap water and distilled water for 2 minutes, with 80% ethyl alcohol then counterstained for 2 minutes with eosin-phloxine solution. Histological observations were made under light microscope.

Statistical Analysis

For each analysis, descriptive statistical processing was used that included the statistic mean (average), standard deviation and the standard error of mean. The Student's *t*-test was applied to evaluate the differences of mean values, the statistic variation, tendency and statistical significance of null hypothesis or the correlation of the pairs of values (one-way ANOVA).

Results

Toxicity study:

The results indicated that different doses of AGHE and AGRE (up to 3000 mg kg^{-1}) did not produce any symptoms of acute toxicity.

Anti-inflammatory activity

In control group, the carrageenan-induced rat paw edema at 3 h was 2.17±0.02 mL (Table 1). The mean reduction in rat paw edema Carrageenan with phenylbutazone (PBZ), was 1.28±0.04 mL. The mean reduction in rat paw edema of herbal extracts AGHE 250 and 500mg/kg was 1.98±0.03 and 1.57±0.03 mL respectively, while root extracts AGRE, 250 and 500mg/kg was 1.72 ± 0.02 and 1.37±0.01 mL respectively.

Table 1: Effect of AGRE and AGHE on carrageenan induced hind paw edema in rats

Percentage inhibition	Net Reduction	Edema volume (ml) after 3h	Edema volume (ml) Before Carrageenan	Dose mg/kg	Treatment
---	1.12±0.02	2.17±0.02	1.05±0.03	0.05ml of 1%	Carrageenan
82.61	0.19±0.001***	1.28±0.04	1.08±0.04	100	PBZ+ Carrageenan
7.28	1.04±0.03	1.98±0.03	0.94±0.04	250	AGHE+ Carrageenan
48.43	0.75±0.06***	1.72±0.02	0.99±0.05	500	AGHE+ Carrageenan
33.66	0.743±0.03***	1.34±0.02	0.98±0.02	250	AGRE+ Carrageenan
64.04	0.40±0.04***	1.37±0.01	0.97±0.04	500	AGRE+ Carrageenan

Values are mean ± SEM. n=5, ***P< 0.001 when compared carrageenan with PBZ+ Carrageenan, AGHE + Carrageenan (250 and 500mg/kg, bw), AGRE + Carrageenan (250 and 500mg/kg, bw). The result was compared by Student's *t*-test and one way ANOVA.

Table 2: Effect of AGHE, AGRE and silymarin on serum activity of ALT, AST, GGT, ALP and bilirubin in CCl_4 intoxicated rats

Bilirubin (mg/dl)	ALP (IU/l)	GGT	ALT (IU/l)	AST (IU/l)	TREATMENT
0.54 ±0.02	294.33 ±6.17	4.70 ±0.28	32.86 ±2.39	74.40 ±13.26	Normal (1ml)
2.34 ±0.16***	514.16 ±11.75 ***	11.25 ±0.37	194.16 ±10.08 ***	211.33 ±10.08***	CCl_4 (1.25mg/kg)
0.90 ±0.06	329.16 ±9.77	5.83 ±0.25	112.93 ±6.13	124.16 ±7.13	Silymarin (10 mg/kg)
2.31 ±0.09	463.00 ± 12.75*	10.53 ±0.24	194.33 ±8.01	212.16 ± 6.75	AGHE+CCl4 (250mg/kg)
2.33 ±0.09	466.33 ±10.34*	11.05 ±0.42	199.33 ±4.78	215.66 ±5.97	AGHE+CCl4 (500mg/kg)
1.27 ± 0.03***	334.83 ±8.82***	7.36 ±0.26***	150.83 ±5.90***	151.16 ±5.99***	AGRE+CCl4 (250mg/kg)
1.06 ±0.05***	329.00 ±4.37***	7.03 ±0.26***	115.43 ±3.19***	128.50 ±3.33***	AGRE+CCl4 (500mg/kg)

Values are mean ± SEM. n=5, *P< 0.05, **P< 0.01 ***P< 0.001, statistically significant compare to normal control group. When compared CCl_4 with Silymarin + CCl_4, AGHE + CCl_4 (250 and 500mg/kg, bw) and AGRE + CCl_4 (250 and 500mg/kg, bw). The result was compared by Student's *t*-test and one way ANOVA.

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Table 3: Effect of AGHE and AGRE on Total Protein, MDA and NP-SH in liver tissue of rats with CCl4 induced-hepatotoxicity

NP-SH (nmol/g)	MDA (nmol/g)	Total protein (g/l)	Dose mg/kg	Treatment
5.86±0.37	1.077±0.05	125.51± 3.54	0.05ml of 1%	Normal Saline
1.10±0.09***	5.10±0.3***	56.55±3.47***		CCl_4
3.66±0.15***	2.04±0.12***	92.41±3.54***	10	Silymarin
1.26±0.12	3.82±0.29*	72.18±4.80***	250	AGHE + CCl_4
1.42±0.12*	4.92±0.20***	85.51±4.88***	500	AGHE + CCl_4
1.34±0.09*	2.17±0.20***	100.68±6.59***	250	AGRE + CCl_4
2.34±0.13***	1.78±0.10***	101.60±4.69***	500	AGRE + CCl_4

Values are mean ± SEM. n=5, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to CCl_4 with Silymarin, AGHE + CCl_4 (250 and 500 mg/kg) and AGRE + CCl_4 (250 and 500 mg/kg). The result was compared by Student's *t*-test and one way ANOVA.

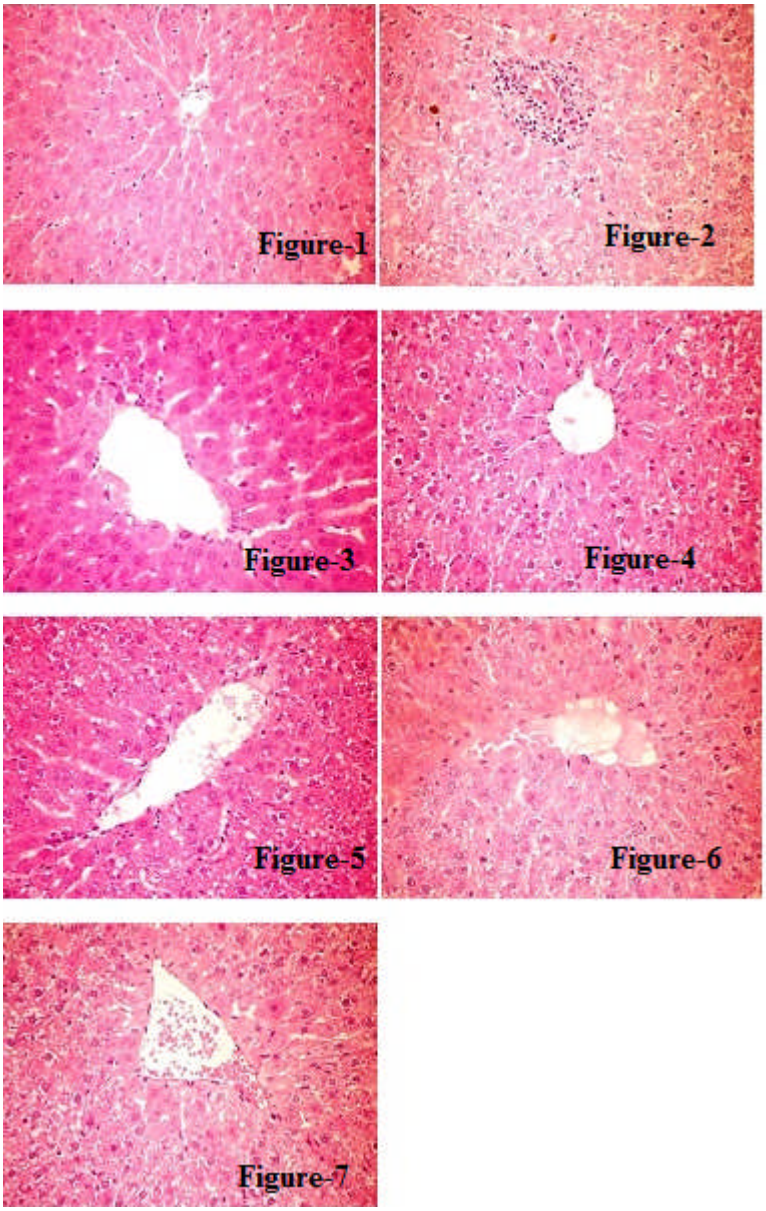

Figure 1-7: Light micrograph of liver sections. Liver section from control rats appeared normal histological structure of hepatic lobule (Figure-1). Liver section from CCl_4 rats appeared hydropic degradation of hepatocytes and partial infiltration with inflammatory cells (Figure-2). Liver section of Silymarin-treated rats appeared slightly activation of kupffer cells (Figure-3). Liver section of CCl_4+AGHE 250mg/kg treated rats showed local hepatic hemorrhage (Figure-4). Liver section of CCl_4+AGHE 500mg/kg treated rats showed no histopathological changes (Figure-5). Liver section of CCl_4+AGRE 250mg/kg treated rats showed slight kupffer cells activation (Figure-6). Liver section of CCl_4+AGRE 500mg/kg treated rats showed slight granulating of the cytoplasm of hepatocytes (Figure-7). Haematoxylin and eosin stain, H and E magnification × 400

Serum Biochemical's Assays

Assays of ALT, AST, GGT, ALP and bilirubin in rat's serum were given in **(**Table 2**)**. CCl_4 (1.25ml/kg), significantly (p<0.001),

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elevated the serum activities of ALT, AST and GGT as compared to the normal saline animals. Administration of AGHE and AGRE at doses of 250 and 500mg/kg prior to CCl_4 significantly protected against the elevation of transaminases, ALP and bilirubin levels. The serum activities of ALT, AST and GGT in rats treated with AGHE at a dose of 250 mg/kg plus CCl_4 were 212.16 ± 6.75, 194.33±8.01 and 10.53±0.24 IU/l, respectively and with 500 mg/kg plus CCl_4 were 215.66±6.97, 199.33±4.78 and 11.05±0.42 IU/l, respectively. These values were not significant when compared with the intoxicated control rats (211.33±10.08, 194.16±7.53 & 11.25±0.37 IU/l respectively). The levels of ALT, AST and GGT in rats received AGRE at a dose of 250 mg/kg plus CCl_4 were 151.16±5.99, 150.83±5.90 and 7.36±0.26 IU/l, respectively and with 500 mg/kg plus CCl_4 were 128.50±3.33, 115.43±3.91 and 7.03±0.26 IU/l, respectively. They were highly significant when compared with the intoxicated control. Similarly, the levels of ALP and bilirubin were non significantly decreased for AGHE, while in AGRE 250 mg/kg plus CCl_4 treated group (334.83±8.82 IU/l & 1.27±0.03 mg/dl respectively) were significantly decreased ($p < 0.01$), when compare with intoxicated control group (514.16±11.75 IU/l & 2.34±0.16 mg/dl, respectively). Comparatively the decrease in case of *AGRE* 500 mg/kg plus CCl_4 treated group (329.83±4.37 IU/l & 1.06±0.05 mg/dl respectively), was highly significant. Silymarin treated animals also prevented the elevation of ALT, -AST, GGT, ALP and bilirubin (124.16±7.13 U/l, 112.93±6.13 U/l, 5.83±0.025 U/l, 329.16±9.77 U/l and 0.90±0.06 mg/dl respectively).

Estimation of total protein

Total liver tissue protein concentration in the AGHE and AGRE treated groups was higher than intoxicated control (56.55± 3.47 g/l). The highest level was recorded with AGRE group 500 mg/kg dose (101.60±4.69g/l) (Table-3).

Malondialdehyde assays

The effect of AGHE and AGRE on the CCl_4-induced lipid per oxidation was examined through observation of the levels of MDA in liver tissues. Hepatic MDA level was significantly ($p < 0.001$), elevated in the CCl_4-intoxicated control group (5.10±0.3 nmol/g tissue) than the normal animals (1.077±0.05 nmol/g tissue). Silymarin (10 mg/kg, i.p.), treatment also prevented the CCl_4 elevatation of MDA (2.04±0.12 nmol/g tissue). Treatment of AGHE (500 mg/kg) and AGRE (250 & 500 mg/kg) with CCl_4 highly significantly ($P<0.001$) prevented the elevated of MDA (Table-3).

NP-SH assays

Rats intoxicated with CCl_4 showed a significant decrease **(**1.10±0.09 nmol/g), in liver NP-SH content as compared to the control (5.86±0.37 nmol/g), rats. Treatment with AGHE 500 mg/kg b.w. along with CCl_4 showed a significant increase ($p<0.05$), in liver NP-SH**.** Meanwhile, treatment with AGRE (either 250 or 500 mg/kg b.w.), along with CCl_4, both of doses, showed a significant increase ($p<0.001$) in liver NP-SH (Table-3)

Histopathological Studies

The microscopic examination of the liver sections obtained from CCl4 intoxicated rats revealed hepatocytes changes associated with hepatotoxicity. The histopathological examination of liver of control and treated animals was summarized in figures 1-6. Livers of the negative control rats showed the normal histological structure of hepatic lobule (Figure 1). The hepatocytes; in CCl_4-intoxicated rats showed hydropic degradation and partial infiltration with inflammatory cells (Figure 2),. Group-III (Silymarin-treated animals), showed local hepatic hemorrhage and slight activation of kupffer cells (Figure 3). Group-IV (CCl_4 + AGHE 250mg/kg animals) showed local hepatic hemorrhage (Figure 4). Group-V (CCl_4 + AGHE 500 mg/kg), showed no histopathological changes (Figure 5). Group-VI (CCl_4 + *AGRE* 250mg/kg) showed slight kupffer cells activation (Figure 6), while Group-VII (CCl_4 + *AGRE* 500mg/kg) showed slight granulating of the cytoplasm of hepatocytes (Figure 7)

Discussion

Doses of AGHE and AGRE (up to 3000 mg kg^{-1}), did not produce any symptoms of acute toxicity in mice. Both extracts are safe for animal use, hence doses of 250 and 500 mg/kg were selected for the experimental work. The edema started between 0 to 2 hrs, and reaches to its maximum level at approximately 3 hrs after the injection of carrageenanm and then began to decline in rat paw (Vinegar et al., 1987). The probable mechanism of action of carrageenan induced edema is bi-phasic; the release of histamine, serotonin, 5-HT and kinins in the first phase; while swelling is related to the release of prostaglandin, bradykinins and lysozymes-like substances in 2-3 hrs, in the second phase (Di Rosa et al., 1971; Brooks and Day, 1991). The related species show the significant anti-inflammatory activities (Ryu et al., 2008). Reduction of carrageenan induced edema by AGHE and AGRE indicated the anti-inflammatory activity of this plant.

Carbon tetrachloride-induced hepatic injury is commonly used as an experimental method for the evaluation of hepatoprotective drugs or medicinal plant extracts (Jamshidzadeh et al., 2005). Generally, the extent of hepatic damage is assessed by histopathological evaluation and the level of cytoplasmic enzymes released into the circulation (Plaa and Charbonneau, 1994). Marked elevation of ALT, AST, GGT, ALP and bilirubin in serum and total protein, MDA and NP-SH in liver tissue indicates damage to the hepatic tissue. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of the membrane (Zimmerman, 1964; Wolf, 1999) that results in raised levels of some known enzymes notably ALT and AST. The normalization of the level of the corresponding enzymes is a definite indication of the hepatoprotective action of the compound under evaluation. Oral administration of AGHE and AGRE remarkably attenuated the increased level of studied enzymes and subsequently recovered the physiological status towards normalization. ALT and AST are the most sensitive markers of hepatocellular injury (Wegwu et al., 2005) and their elevation in serum is indicative of cellular leakage and loss of the functional integrity of cell membranes in liver (Rajesh and Latha, 2004). Decline in ALT, AST and GGT levels after AGHE and AGRE administration indicated improvement in cellular integrity and status of hepatic cells. ALP is a membrane bound enzyme involved in active transport across the capillary wall. The increased level of ALP is also a reliable marker of liver damage (Muriel and Escobar, 2003). GGT is important in transporting amino acids required for the synthesis of GSH in cells. Diminishment in GGT and ALP after AGHE and AGRE treatment is also indicative of its membrane stabilizing activity. Bilirubin is an important degradation product of hemoglobin and is normally excreted into the bile. If hepatic parenchymal damage is severe, less bilirubin will be excreted and hyperbilirubinemia is observed (Klaassen and Watkins, 1984).

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Increase in total serum bilirubin concentration after CCl_4 administration might be attributed to the failure of normal uptake, conjugation and excretion by the damaged hepatic parenchyma, the increased TBARS after CCl_4 administration suggests enhanced LPO due to formation of excessive free radicals and failure of antioxidant defense mechanism leading to tissue damage. The phenolic compounds are known to exert protective effect against CCl_4 intoxication by reducing the MDA production, which is indicative of its antioxidant activity (Fan et al., 2009). Non-protein sulfhydryls are known to be involved in several defense processes against oxidative damage; protect cells against free radicals peroxides and various poisonous substances (Sies, 1999). Thus, a deficiency of GSH within the living organisms can cause tissue injury and malfunction (Ganie et al., 2011). In the current study, the liver NP-SH level in CCl_4-treated groups was significantly diminished when compared with the control group. These findings are in accordance with earlier reports as sulfhydryl levels were significantly depleted in different organs of rats, when exposed to CCl_4 (Ohta et al., 2000). Administration of AGHE and AGRE extracts could manage biochemical changes indicating its protective role in liver tissue. This was supported by alteration in the biochemical markers through CCl_4 intoxication prevented by AGHE and AGRE with CCl_4. The previous reports of related species were proved to be active against carbon tetrachloride (Jia et al., 2012). The results also prove that AGRE is comparatively more active than AGHE. Antioxidant mechanism could be an ameliorative factor in the protective effect of AGRE for CCl_4-induced hepatotoxicity in rats. CCl_4 is a well-known hepatotoxicant model that is activated by CYP system and initiates oxidative and biochemical stress that ultimately damage liver and other tissues, including kidney, heart, lung, testis, brain and blood (Szymonik-Lesiuk et al., 2003).

The phytochemicals triterpenoidal glucuronides obtained from several related species, show hepatoprotective and antioxidant activities (Jia et al., 2012; Bian et al., 2009). The related species possesses highly active anti-inflammatory and hepatoprotective polysaccharides and cycloartane-type saponins (Lee et al., 2013), Macrophyllosaponin B, Astragaloside VII, Calycosin and formononetin (Nalbantsoy et al., 2011). The antioxidant and hepatoprotective phytochemicals present in these related plant supported the present activities. Histopathological observations after CCl_4-administration showed severe damage in hepatocytes, which, basically, supported the alterations observed in biochemical assays. Centrilobular necrosis, ballooning of hepatocytes, infiltration of lymphocytes and steatosis of liver cells were characteristic alterations occurred due to CCl_4 intoxication (Shukla et al., 2005; Bhadauria et al., 2007). Treatment of AGHE (250mg/kg bw,) plus CCl_4 showed focal hepatic haemorrhage, while AGHE (500mg/kg bw,) plus CCl_4 showed no histopathological changes. AGRE (250mg/kg bw,) plus CCl_4 showed slight kupffer cells activation, while AGHE (500mg/kg bw,), plus CCl_4 showed slight granularity of the cytoplasm of hepatocytes. These histopathological changes indicated regenerative effects of AGHE and AGRE. This can be considered as an expression of the functional improvement of hepatocytes, which might be due to accelerated regeneration or limited damage in the presence of *A. gummifer*.

Conclusion

Treatment of AGRE and AGHE significantly reduced carrageenan induced edema and reversed the CCl_4-induced enzymatic, oxidative and histopathological alterations. Comparatively, the activity of AGRE is highly significant when compared with AGHE.

Conflict of Interests: There is no conflict of interests.

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