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# ANTI-INFLAMMATORY AND HEPATOPROTECTIVE STUDY OF *ASTRAGALUS PLUMOSUS* VAR. *AKARDAGICUS* AND *ASTRAGALUS LAMARCKI* (FAMILY: FABACEAE) IN WISTAR ALBINO RATS

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**ABSTRACT**

The present study aims to evaluate the anti-inflammatory and hepatoprotective effects of the alcoholic extract of *Astragalus plumosus* (ApE) and *Astragalus lamarcki* (AlE) against carrageenan induced inflammation and carbon tetrachloride ($CCl_4$)-induced liver injury, respectively**.** To evaluate the anti-inflammatory effects of ApE and AlE, 30 male rats were divided into 6 equal groups.Paw edema was induced by injection of 0.1 ml carrageenan in normal saline (1%, w/v) into the subplantar region of the hind paw of rats. The volume of paw edema was attenuated byoral administration of ApE and AlE. For hepatoprotective effects, 35 rats were equally divided into 7 groups.The hepatotoxicity, induced by a single dose of $CCl_4$, produced significantly increased levels of serum AST, ALT, GGT, ALP and total bilirubin. In addition, decreased total proteins (TPs), increased malondialdehyde (MDA), decreased non-protein sulfhydryls (NP-SH) activities were observed in the hepatic tissues. Pre-medication of $CCl_4$-intoxicated rats with ApE and AlE at doses of 250 and 500 mg/kg reversed the abnormal liver diagnostic stricture. The results showed that, *Astragalus plumosus* extract (ApE) was anon-significant anti-inflammatory agent, whereas *Astragalus lamarcki* extract (AlE) was a highly significant anti-inflammatory agent. Both extracts showed a significant hepatoprotective activity. As per our knowledge, there is no previous report on these plants. Further preservative studies are required in support of present findings.

**Keywords: *Astragalus plumosus*, *Astragalus lamarcki*, Fabaceae, Anti-inflammatory, Hepatoprotective, Carbon tetrachloride, Silymarin**

## INTRODUCTION

Liver damage from anti-inflammatory drugs are rare, but these medications should not be used in persons with cirrhotic liver diseases because of bleeding problems and renal failure due to decrease prostaglandin-mediated blood flow to the kidneys leading to an increased risk of renal failure in persons with cirrhosis **[1-2]**. Drugs are an important cause of liver injury. More than 900 drugs, toxins, and herbs have been reported to cause liver injury. Drugs account for 20-40% of all instances of hepatic failure **[3]**. Chronic liver cirrhosis and drug induced liver injury are accounting the ninth leading cause of death in western and developing countries **[4]**. The main causative factors for the liver diseases in the developed countries are excessive alcohol consumption, and viral-induced chronic liver diseases, while in the developing countries the most common causes are parasitic disease, hepatitis B and C viruses, environmental toxins and hepatotoxic drugs **[5]**. A number of herbs show promising hepatoprotective activity, including Silymarin, *Andrographi spaniculata, Azadirachta indica, Cassia fistula, Glycyrrhiza glabra, Hibiscus rosasinensis, Phyllanthus debilis, Phyllantus amarus, Picrorrhiza kurroa, Sida veronicaefolia* and some herbal combinations from India, China and Japan **[6-7]**. Family Fabaceae is considered as one of the major plant families in the world including approximately 269 genera and 5100 species, out of which, approximately more than three thousands *Astragalus* species found all over the world **[8-9]**. It is mostly diverse in the Irano-Turkish region of southwestern Asia, the Sino-Himalayan Plateau of South Central Asia, the Central Asia region and the Great Basin, and the Colorado Plateau of western North America **[10-11]**. Some of these *Astragalus* species are used in traditional medicine for the treatment of various diseases for example, diabetes mellitus, nephritis and cancer because of their antioxidant activities that protect cells against the damage of free radicals and biological activities such as antiviral, antibacterial, anti-inflammatory and diuretic activities **[12-18]**. Over the years, a large number of publications have been brought out on genus *Astragalus*, both on regional and global. Astragalus has shown some vasodilatory as well as anti-inflammatory action. Its anti-inflammatory effects occur, seemingly, because it inhibits the release of histamines from mast cells. Consequently, *Astragalus* could help relieve hay fever and other allergic conditions **[19]**. The sixteen cycloartane glycosides have been isolated from the methanol extracts of *Astragalus plumosus* var. *krugianus*, among which

krugianoside A, prevented the elevation of ROS induced by t-BOOH, suggesting the potential activity of this compound to protect fibroblasts from oxidative stress **[20]**. Antimicrobial, Anti-inflammatory, analgesic, antipyretic, modulates immune functions and antioxidant activities of some of *Astragalus* species, were already reported **[21-22]**. *Radix Astragalus* mainly contains saponins, flavone, polysaccharides and other chemical constituents **[23]**. In addition, some investigations have reported that *Astragalus* polysaccharides (APS) had hepatopretective effects on liver injury induced by $CCl_4$ in mice. The hepatoprotective activity of the ethanol extract of *Astragalus kahiricus* (Fabaceae) roots against ethanol-induced liver apoptosis was evaluated and it showed very promising hepatoprotection **[24]**. In spite of that the anti-inflammatory and antioxidants properties of *A. plumosus* and *A. lamarcki*, growing in the eastern part of Turkey, have been previously investigated. This is the first report onthe anti-inflammatory and hepatoprotectiveactivities of the alcoholic extracts of these species.

## MATERIALS AND METHODS

### Chemicals and Reagents

All the chemicals and reagents usedwere analytical grade. Carrageenan was purchased from BDH Chemicals Ltd., UK, while Phenylbutazone **(**PBZ**)**, Silymarin, Lipid Peroxidation (MDA) Assay Kit, EDTA, Trichloroacetic acid (TCA),and 5-5'-dithiobis-(2 nitrobenzoic acid) DNTB were purchased from Sigma Aldrich.

### Collection and Authentication of Plant

*A. plumosus* var. *akardagicus* and *A. lamarcki* (Fabaceae) whole plants were collected in June 25$^{th}$ 2010, from Elazig-Hazargolu road, 23 km southeast of Elazig, East Anatolia, Turkey. A voucher specimen has been deposited in Herbarium of the Pharmaceutical Botany Department, Faculty of Pharmacy, Hacettepe University, Ankara Turkey (HUEF 10-23). The plant was authenticated by Prof. Dr. Zeki Aytac, Gazi University, Department of Biology, Faculty of Science and Art, Ankara, Turkey.

### Plant Extraction

The air-dried powdered herbs were extracted with 80% aqueous EtOH under reflux for 4 h then the extract was filtered. The filtrate was subjected to rotary evaporation (BUCHI-R 215; 40°). The resinous extracts of *A. plumosus* var. *akardagicus* (ApE) and *A. lamarcki* (AlE) herbs were lyophilized using freeze drier. The ApE and AlE extracts were obtained with yields of 15 and 17.5 % respectively, and were stored in a freezer at -80°C until used for the experimental studies.

### Methods

### Animals

Wistar albino rats (200-230g) of either sex, obtained from the experimental animal care

center, college of Pharmacy, King Saud University, Riyadh, were housed under constant temperature ($22\pm2^0$C), humidity (55%) and 12 hr light/dark condition. They were provided with Purina chow diet and drinking water *ad libitum*.

**Determination of Acute Toxicity and Median Lethal Dose ($LD_{50}$) of Extracts**

Rats were divided into groups of 5 and the tested extracts were administered orally in doses of 100 to 3000 mg/kg body weight. Number of death and signs of acute toxicity per dose within 24 h were recorded and the $LD_{50}$measured using the method described by Lorke **[25]**.

**Carrageenan-Induced Paw Edema in Rats**

Pedal inflammation was produced in albino rats according to the method **[26]**. The test groups of rats were treated, orally, with ApE (250 and 500 mg/ kg) and AlE (250 and 500 mg/ kg). After one hour, acute inflammation was produced by the sub planterinjection of 0.1 ml of (1%, w/v) carrageenan in normal saline in the right hind paw of each rat. At the same time the reference group was given an aqueous solution of phenylbutazone (100 mg/kg) and control group was given 5ml/kg of normal saline. The displacement technique was used for the measurement of paw volume at 0 h, 2 h and 3 h after carrageenan injection using a Plethysmometer (Apelex, France). The inhibitory activity was calculated according to the following formula

**Inhibitory activity = 100(1-a-x/b-y)**

Where "*a*" is the mean foot paw volume of the rats after the administration of carrageenan in test and reference groups; "x" is the mean foot paw volume of the rats before the administration of carrageenan in test and reference group; "*b*" is the mean foot volume of the rats after the administration of carrageenan in the control group; "*y*" is the mean foot volume of the rats before the administration of carrageenan in the control group.

**Hepatoprotective Study**

The Hepatoprotective activity was evaluated in Wistar albino rats using $CCl_4$ induced liver injury according to the method **[27]**. The rats were divided into Seven groups (n=5). Group (1) served as control (normal saline), Group (2) served as $CCl_4$-intoxicated, Group (3) served as positive control (Silymarin). Groups (4) & (5) served as (250 and 500 mg/kg b.w.) ApE treated groups, while Groups (6) & (7) served as (250 and 500 mg/kg b.w.) AlEtreated groups. All 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 with cold saline, blotted with filter paper and weighed. Serum was separated by centrifugation of the blood at

3000 rpm/ 4°C for 10 min. 10% Liver homogenate was prepared in sucrose (0.25M) solution and centrifuged (7000 rpm for 10 min at 4ºC). The supernatant of centrifuged solution was used for biochemical assays. For the histopathological analysis, ventral portion of the left lateral liver lobe were collected and fixed in 10% neutral-buffered formalin.

**Biochemical Assays**

The collected serum was used for the measurement of biochemical parameters including, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyltranspeptidase (GGT) and total bilirubin **[28]**. Protein concentration was estimated according to the method **[29]** using bovine serum albumin (BSA) as a standard.

**Determination of Malondialdehyde (MDA)**

The measurement of Malondialdehyde (MDA) was used as an indicator of lipid peroxidation **[30]**. In brief, 10% (w/v) liver was homogenate and 500 µl phosphate buffer (100mM, pH-7.4) and reaction mixture was incubated for 1h at 37 $^{o}$C. 2 ml of the supernatant were mixed with 2 ml of 67% 2-thiobarbituric acid and 1 ml of 20% trichloroacetic acid solution and heated in water bath (95 $^{o}$C) for 30 min. Thereafter cooling the mixture was centrifuged at 300 rpm for 15 min and the supernatant was collected. Amount of TBARS formed was measuredby taking the absorbance of the supernatant at 532 nm (SHIMADZU, UV-1800). The result was measured TBARS concentrations and expressed as MDA nmol/mg protein.

**Estimation of Non-Protein Sulfhydryls (NP-SH)**

The Non-Protein sulfhydryls (NP-SH) were measured according to the method **[31].**The liver was homogenized in the ice cold 0.02 mmol/l ethylene diaminetetra acetic acid (EDTA). Aliquots of 5 ml of the homogenate were mixed in 15 ml test tube with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid (TCA). The homogenate was centrifuged at 3000 rpm.Total of 0.1 ml of supernatant was suspended in tris buffer, 5-5'-dithiobis-(2 nitrobenzoic acid) (DTNB) and absorbance was measured within 5min at 412 nm against reagent blank with no homogenate.

**Histopathological Studies**

A small fragment of liver tissue was placed in 10% formalin (diluted to 10% with normal saline) for 1 hr according to the method **[32]**. The tissue was dehydrated by ascending grades of isopropyl alcohol by immersing in 80% isopropanol overnight followed by100% isopropyl alcohol for 1 hour and finally four times paraffin wax for 1h. The tissue was transferred into paraffin

waxe filled molds. A tissue section (3 μm) was prepared using rotary microtome (Leitz1512, GMI Inc.). The section was, afterwards, placed on clean slide and placed onto warming table at 37-40°C. The slide was then stained for 15min with Mayer's hematoxylin solution, and washed for 15 min in lukewarm running tap water and distilled water, followed by 2 min in80% ethyl alcohol then counterstained for 2 min with eosin-phloxine solution. Histological observations were made under light microscope at 400xmagnification.

**Statistical Analysis**

The statistic mean (average), standard deviation and the standard error of mean were calculated using micrsoft excel. The significance of differences was calculated by analysis of variances (ANOVA) and Student's *t*-test using Graph Pad Prism Software (GraphPad, San Diego, California, USA).

**RESULT**

**Acute Toxicity**

*Astragalus plumosus* var. *akardagicus* (ApE) and *Astragalus lamarcki* (AlE) (up to 3000mg/kg) did not produce any symptoms of acute toxicity.

**Anti-inflammatory Activity**

Carrageenan produced paw edema in control ratsafter 3 h of subplanter injection (2.17±0.02 mL). Pretreatment with phenylbutazone (PBZ) resulted in82.61% reduction in the volume of rat paw edema. The percentage reduction in rat paw edema of ApE(250 and 500mg/kg)was less than 9%, while AlE(250 and 500mg/kg)inducedmore than 50% edema reduction (Table1).

**Serum Biochemical Assays**

Carbon tetrachloride (1.25ml/kg) significantly elevated the serum levels of AST (211.33±10.08), ALT (194.16±10.08), GGT (11.25±0.37), ALP (514.16±11.75) and bilirubin (2.34±0.16) when compared to the normal saline animals. Silymarin treatment protected against the elevation of ALT (124.16±7.13 U/l), AST (112.93±6.13 U/l), GGT (5.83±0.025 U/l), ALP (329.16±9.77 U/l) and bilirubin (0.90±0.06 mg/dl). Administration of ApE and AlE at doses of 250 mg/kg and 500mg/kg prior to $CCl_4$ significantly protected against the elevation of AST, ALT, GGT, ALP and bilirubin levels. The serum activities of AST, ALT, and GGT in rats treated with ApE at a dose of 250 mg/kg plus $CCl_4$ were 185.66±7.26, 165.00±7.75 and 10.52±0.53 IU/l, respectively were not significant while activities at dose of 500 mg/kg plus $CCl_4$ were 159.00±4.19, 138.50±3.78 and 8.60±0.25 IU/l, respectively and were more significant when compared with the intoxicated control rats. The rats treated with 250 mg/kg AlE plus $CCl_4$ showed significantly lower AST, ALT and GGT

levels as compared to 500 mg /kg AlE plus $CCl_4$ test group. Similarly, the levels of ALP and bilirubin were only significantly decreased at dose of 500mg /kg, while in AlE both the doses of 250 mg/kg and 500 mg /kg plus $CCl_4$ treated group were significantly decreased when compare with intoxicated control group. Comparatively the decrease in case of AlE at 500 mg/kg plus $CCl_4$ treated group was highly significant.

**Estimation of Total Protein**

Total liver tissue protein concentrations in the AlE treated groups were higher than in intoxicated control (56.55± 3.47 g/l). The tissue protein concentration in ApE treated groups was insignificant at lower dose and less significant at higher dose ($p<0.05$). Both of the AlE groups animals showed highly significant ($p<0.001$) increase in tissue protein concentration.

**Malondialdehyde Assays**

The effect of ApE and AlE on the $CCl_4$-induced lipid peroxidation 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 in the normal animals (1.077±0.05 nmol/g tissue). Silymarin (10 mg/kg, i.p.) treatment also prevented the $CCl_4$ elevation of MDA (2.04±0.12 nmol/g tissue). Treatment with ApE (250 &500 mg/kg) prevented the $CCl_4$ elevation of MDA (4.01±0.18 & 3.19±0.12 nmol/g respectively) less significantly than treatment with AlE (250 & 500 mg/kg) with $CCl_4$ (2.91±0.15 & 2.37±0.14 nmol/g respectively) **(Table3)**.

**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 ApE, 250 and 500 mg/kg b.w. along with $CCl_4$ showed a significant increase ($p<0.01$ & $p<0.001$) in liver NP-SH**.** Meanwhile, treatment with AlE, at 250 or 500 mg/kg b.w. along with $CCl_4$, showed a comparatively less significant increase in liver NP-SH **(Table3)**

**Histopathological Studies**

The microscopic examination of the liver sections obtained from $CCl_4$ intoxicated rats revealed cytoplasmic vacuolization of hepatocytes and partial infiltration with inflammatory cells. Results from the histopathological examination of liver of control and treated animals are summarized in **Figures 1-7**.

**DISCUSSION**

The results of our toxicity studies indicated that the selected doses of both plants extracts were suitable for preliminary evaluation of their potential anti-inflammatory and hepatoprotective

activities. Carrageenan-induced paw edema in rats is known as a sensitive method for studying non-steroidal anti-inflammatory agents and shows a biphasic event which is attributed to the different mediators. Within the first 2 h after carrageenan injection, mainly hyperemia is induced because of the release of histamine and serotonin, whereas prostaglandins and bradykinin potentiate the second phase of edema by mobilization of leukocytes **[33]**.The inflammation was clearly produced at 3 h with carrageenan. The results indicated that ApE did not show significant anti-inflammatory activity whereas AlE showed highly significant activity.These finding revealed that only *A. lamarcki* was an anti-inflammatory plant. This is supported by presence of steroids, flavonoids, saponins, tannins and anthraquinones types of phytochemicals **[34-35]** which could be responsible for the anti-inflammatory activities. The related species *A. membranaceus* and *A. radix* were previously reported for their anti-inflammatory effect due to presence of saponins and polysachharides **[36-38]**.

The $CCl_4$ hepatic injury is commonly used as an experimental method for the evaluation of hepatoprotective drugs or medicinal plant extracts **[39]**. Generally, the extent of hepatic damage is assessed by the level of cytoplasmic enzymes released into the circulation and histopathological evaluation **[40]**. In this study $CCl_4$ induced significant elevation of AST, ALT, GGT, ALP and total bilirubin levels in serum in addition to elevation of MDA and depletion of total protein and NP-SH in liver tissues, indicating damage to the hepatic tissue. ALT and AST are the most sensitive markers of hepatocellular injury **[41]**.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 resulting in raised levels of enzymes notably ALT and AST **[42]**.Oral treatment with ApE and AlE prior to $CCl_4$ significantly protected the elevation of transaminases, indicating improvement in cellular integrity and status of hepatic cells. The increased level of GGT and ALP is also a reliable marker of liver damage **[43]**. Diminishment in GGT and ALP after ApE and AlE treatment is also indicative of its membrane stabilizing activity. 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. Increased total bilirubin due to $CCl_4$ intoxication was reduced after ApE and AlE treatment, indicating its normal uptake, conjugation and excretion. Because albumin is made in the liver, its level tends to drop with liver damage. Albumin has

been widely used in patients with liver damage in an attempt to improve circulatory and renal functions **[44]**. Oral administration of ApE showed little improvement of total protein whereas AlE showed a highly significant improvement as compared to silymarin. The increased Thiobarbituric acid-reactive substances (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.$CCl_4$ 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 **[45]**. Phenolic compounds are known to exert protective effect against $CCl_4$ intoxication by reducing the MDA production, which is indicative of its antioxidant activity **[46]**. Antioxidant mechanism could be an ameliorative factor in the protective effect of ApE and AlE for $CCl_4$-induced hepatotoxicity in rats, and may be due to the presence of phenolic compounds **[47]**. Non-protein sulfhydryls are known to be involved in several defense processes against oxidative damage; they protect cells against free radicals peroxides and various poisonous substances **[48]**. Thus, a deficiency in GSH within living organisms can cause tissue injury and malfunction **[49]**. 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 $CCl_4$ **[50]**. Administration of ApE could manage NP-SH biochemical changes indicating its protective role in liver tissue. Administration of AIE at lower dose did not show any significant alterationin NP-SH biochemical changes while at higher it showed less significant alteration. These results indicate the protective role of doses ApE due to NP-SH changes. A previous report on related Astragalus species was proved to be active against carbon tetrachloride-induced changes **[51]**. The phytochemicals triterpenoid and glucuronides obtained from several related species also showed hepatoprotective and antioxidant activities **[52]**. Bioactive secondary metabolites such as polysaccharides and cycloartane-type saponins, Macrophyllosaponin B, Astragaloside VII, Calycosin and formononet in that possess significant hepatoprotective activities were also extracted from these species **[53-54]**. 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 **[55-56].**Treatment with ApE and Silymarin showed slight activation of kupffer cells, while treatment with AlE showed hydropic degeneration and vacuolization of some hepatocytes. The histopathological **Figures (4 & 7)** suggest the protective nature of these extracts. The results obtained with *A. plumosus* var. *akardagicus* extract (ApE) indicated no anti-inflammatory activity. However, it showed significant normalization of AST, ALT, GGT, ALP, Bilirubin, MDA, NPSH and histopathological evaluation indicating its hepatoprotective. On the other hand, results with *Astragalus lamarcki* extracts (AlE) indicated anti-inflammatory and hepatoprotective potencies, in addition to significant normalization of AST, ALT, GGT, ALP, Bilirubin, total protein, and MDA.

**CONFLICT OF INTERESTS**

There is no conflict of interest.

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**Table 1: Effect of ApE and AlE on Carrageenan Induced Hind Paw Edema in Rats**

| Treatment | Dose mg/kg | Increased in rat paw edema (ml±SEM) | | Net Reduction | Percentage inhibition |
|---|---|---|---|---|---|
| | | 0h | 2h | | |
| Carrageenan (1%) | 0.1 ml/kg | 1.05±0.03 | 2.17±0.02 | 1.12±0.02 | --- |
| PBZ+ Carrageenan | 100 | 1.08±0.04 | 1.28±0.04 | 0.19±0.001*** | 82.61 |
| ApE+ Carrageenan | 250 | 1.05±0.04 | 2.07±0.03 | 1.02±0.04 | 8.76 |
| ApE + Carrageenan | 500 | 0.86±0.05 | 1.88±0.04 | 1.13±0.01 | |
| AlE+ Carrageenan | 250 | 1.11±0.01 | 1.62±0.03 | 0.51±0.03*** | 53.78 |
| AlE + Carrageenan | 500 | 1.07±0.04 | 1.73±0.03 | 0.55±0.05*** | 50.52 |

**Values are Expressed as Mean ± SEM. n=5 rats/ group, ***P< 0.001 When Compared Carrageenan With PBZ+ Carrageenan, ApE + Carrageenan (250 and 500mg/kg, bw), AlE + Carrageenan (250 and 500mg/Kg, Bw), The Result Was Compared By Student's *T*-Test And One Way Anova, Using Graphpad Prism 5 Software**

**Table 2: Effect of ApE, AlEandsilymarin on Serum Activity of ALT, AST, GGT, ALP and Bilirubin in $CCl_4$-Intoxicated Rats**

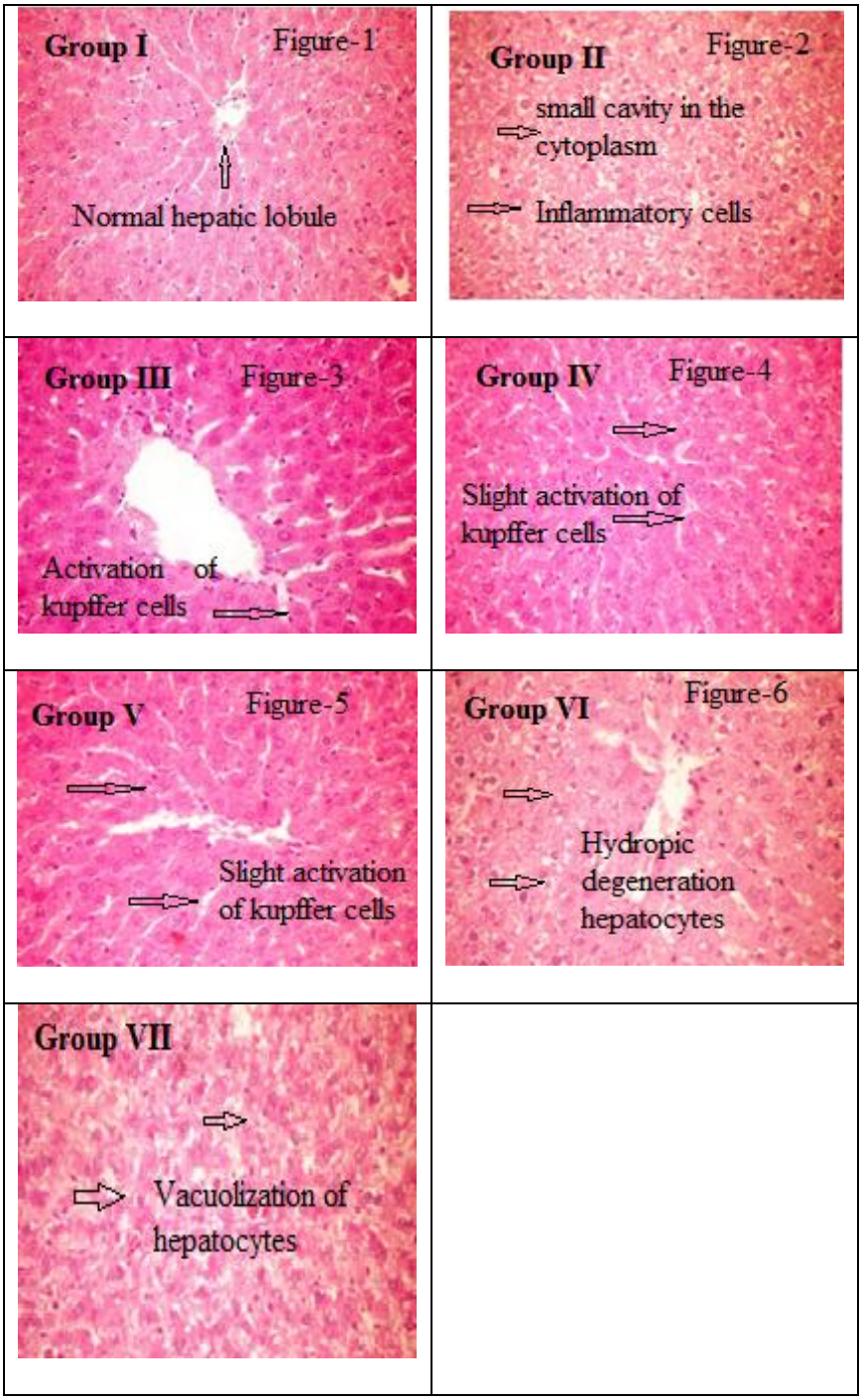
| Group | Treatment | AST (IU/l) | ALT (IU/l) | GGT | ALP (IU/l) | Bilirubin (mg/dl) |
|---|---|---|---|---|---|---|
| Group (1) | Normal saline (2ml/kg, p.o.) | 74.40 ±13.26 | 32.86 ±2.39 | 4.70 ±0.28 | 294.33 ±6.17 | 0.54 ±0.02 |
| Group (2) | $CCl_4$ (1.25ml/kg,i.p.) | 211.33 ±10.08*** | 194.16 ±10.08*** | 11.25 ±0.37*** | 514.16 ±11.75*** | 2.34 ±0.16*** |
| Group (3) | Silymarin (10 mg/kg, i.p.) | 124.16 ±7.13*** | 112.93 ±6.13*** | 5.83 ±0.25*** | 329.16 ±9.77*** | 0.90 ±0.06*** |
| Group (4) | ApE+CCl4 (250mg/kg, p.o.) | 185.66 ± 7.26 | 165.00 ±7.75 | 10.52 ±0.53 | 467.16 ± 19.66 | 2.06 ±0.06* |
| Group (5) | ApE+CCl4 (500mg/kg, p.o.) | 159.00 ±4.19*** | 138.50 ±3.78*** | 8.60 ±0.25*** | 445.16 ±11.83** | 1.52 ±0.05*** |
| Group (6) | AlE+CCl4 (250mg/kg) | 177.00 ±4.51* | 151.83 ±5.39*** | 9.73 ±0.35* | 460.66 ±15.22* | 1.70 ± 0.07*** |
| Group (7) | AlE+CCl4 (500mg/kg, p.o.) | 146.16 ±4.51*** | 137.50 ±8.22*** | 7.68 ±0.25*** | 358.83 ±8.79*** | 1.55 ±0.04*** |

**NOTE: 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) Showed Statistically Significant When the Result was Compared by Student's *t*-test and Oneway ANOVA, Using Graphpad Prism 5 Software**

**Table 3: Effect of ApE and AlE on Total Protein, MDA and NP-SH in Liver Tissue of Rats with CCl4 Induced-Hepatotoxicity**

| Treatment | Total protein (g/l) | MDA (nmol/g) | NP-SH (nmol/g) |
|---|---|---|---|
| Normal saline (2ml/kg, p.o.) | 125.51± 3.54 | 1.077±0.05 | 5.86±0.37 |
| $CCl_4$ (1.25ml/kg,i.p.) | 56.55±3.47*** | 5.10±0.3*** | 1.10±0.09*** |
| Silymarin (10 mg/kg, i.p.) | 92.41±3.54*** | 2.04±0.12*** | 3.66±0.15*** |
| ApE+CCl4 (250mg/kg, p.o.) | 64.36±3.81 | 4.01±0.18* | 1.55±0.10** |
| ApE+CCl4 (500mg/kg, p.o.) | 72.18±3.92* | 3.19±0.12*** | 1.81±0.10*** |
| AlE+CCl4 (250mg/kg) | 80.00±3.10*** | 2.91±0.15*** | 1.12±0.05 |
| AlE+CCl4 (500mg/kg, p.o.) | 94.71±4.42*** | 2.37±0.14*** | 1.44±0.13* |

**Values are Expressed as Mean ± SEM. n=5 Rats/ Group,*P< 0.05, **P< 0.01, ***P< 0.001 When Compared to $CCl_4$ with Silymarin, *ApE* + $CCl_4$ (250 and 500 mg/kg) and *AlE* + $CCl_4$ (250 and 500 mg/kg). The result was Compared by Student's *t*-test and Oneway ANOVA, Using Graphpad Prism 5, Software**




**Figures 1-7: Histopathological Section of the Liver Tissues (H & E×400) of Albino Wistar Rats Treated; Figure-1, 2, 3 Showed the Tissues of Rat Treated with Normal Saline, $CCl_4$ and Silymarin Respectively; Figure 4 and 5 Showed the Tissues of Rats Treated with 250 and 500 mg/kg ApE Respectively, while Figure 6 and 7 Showed the Tissues of Rats Treated with 250, 500 mg/kg AIE Respectively**