HEPATOPROTECTIVE ACTIVITY OF *CHENOPODIUM ALBUM* LINN. AGAINST PARACETAMOL INDUCED LIVER DAMAGE

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Summary

Dried aerial parts of *Chenopodium album* Linn. (Chenopodiaceae) are used in variety of diseases in traditional Indian system of medicine including hepatic ailments.The aim of present study was to validate hepatoprotective activity of aerial parts of *Chenopodium album* Linn using paracetamol as hepatotoxin. Alcoholic [ALCA] and aqueous [AQCA] extracts of the aerial parts of *Chenopodium album* at the doses of 200 and 400 mg/Kg were evaluated for hepatoprotective activity against paracetamol induced hepatotoxicity using biochemical markers and by histopathological method. The aqueous extract at a dose of 400 mg/kg was found to be more potent when compared to Silymarin. ALCA and AQCA [200 & 400 mg/Kg] showed significant hepatoprotective activity against paracetamol induced hepatotoxicity as evident by restoration of serum transaminases, alkaline phosphatase and bilirubin content. Histopathology of the liver tissue further confirmed the reversal of damage induced by hepatotoxin.Present study showed that the alcoholic and aqueous extracts of *Chenopodium album* significantly restore physiological integrity of hepatocytes. Aqueous and alcoholic extract did not show any sign of toxicity up to oral dose of 5 g/Kg in mice.

Key words: *Chenopodium album* Linn; hepatoprotective activity; paracetamol; serum biochemical assessment; histopathology

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Introduction

Liver diseases have become a global concern worldwide. The principal causative factor is increasing alcohol consumption, infection, malnutrition, anemia and availability of hepatotoxic drugs over the counter. Treatment options for common liver diseases such as cirrhoses, fatty liver and chronic hepatitis are problematic. The conventional drugs used in the treatment of liver diseases viz., corticosteroids, antiviral and immunosuppressant agents are sometimes inadequate and may lead to serious adverse effects. Paradoxically, these may themselves cause hepatic damage. Eg: cholestatic jaundice with azathioprine and elevation of serum transaminases by interferon and virazole[1]. It is therefore imperative to search alternative drugs for the treatment of liver disease to replace the currently used drugs of doubtful efficacy and safety[2]. In absence of a reliable liver protective drug in the modern system of medicine, a number of medicinal preparations in Ayurveda, the Indian system of medicine, are recommended for the treatment of liver disorders. Natural remedies from medicinal plants are considered to be effective and safe alternative treatments for hepatotoxicity.

In such context, one such drug is *Chenopodium album* Linn which has ethno pharmacological relevance to be used for hepatic disorders[3]. *Chenopodium album* Linn (Chenopodiaceae) found wild up to an altitude of 4700 m and cultivated throughout India particularly Western Rajasthan, Kulu valley and Shimla. It is commonly known as Lamb's quarte, wild spinach, white goosefoot in English[4,5]. In Tradition System of Medicine, it is used as an anthelmintic,antidiarrhoeal, antiphlogistic, antirheumatic, contraceptive, odontalgic, laxative, cardiotonic, antiscorbutic, blood purifier, hepatic disorder, spleen enlargement, biliousness, intestinal ulcers, digestive, carminative, aphrodisiac, dyspepsia, flatulence, strangury, seminal weakness, pharyngopathy, splenopathy, hemorrhoids, ophthalamopathy, cardiac disorder and general debility[6-9]. The phytoconstituents isolated so far from the plant are ascorbic acid, β-carotene, catechin, gallocatechin, caffeic acid, p-coumaric acid, ferulic acid, β-sitosterol, campesterol, xanthotoxin, stigmasterol, n-triacontanol, imperatorin, ecdysteroid[10], cinnamic acid amide alkaloid[11], phenol, saponin, apocartenoids[12], crytomeridiol[13], n-trans-feruloyl-4-O-methyl dopamine and syringaresinol[14] and β-sitosterol, lupeol and 3 hydroxy nonadecyl henicosanoate[15].The pharmacological activity reported so far from this plant are antipruritic and antinociceptive activity[16], anthelmintic activity[17] and as vaginal contraceptive[18] .

As there is not report on hepatoprotective activity, this prompted us to investigate the hepatoprotective activity of aerial parts of *Chenopodium album* extracts.

Materials and Methods

Plant Material

Plant material used in the study consisted of aerial parts of *Chenopodium album Linn*, collected from the local area of Nadaun, Distt. Hamirpur (H.P.), and authenticated by Dr. Sushil Vashi, Reader, Department of Botany, Govt Degree College of Arts, Commerce and Science, Hamirpur (H.P.). A voucher specimen is preserved in the Department.

Preparation of plant extract:

Crude aerial parts of *Chenopodium album* were subjected to pulverizations and passed through sieve no. 40. The powder [300 g] was packed into a soxhlet apparatus and extracted with petroleum ether (60-80º C) for 18 h. The same marc was successively extracted with alcohol and afterwards with distilled water for 18 hours. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by rotary vacuum evaporator and evaporated to dryness and the percentage yield was found to be 2.3, 0.6 and 15.3 % w/w respectively.

Chemicals

Silymarin (Sigma Chemicals, USA), alcohol (CDH, Mumbai), and Paracetamol (Lupin Lab Ltd, Bhopal) were purchased. Other chemicals and reagents used for extraction were of AR grade. Biochemical kits like AST, ALT, ALP, albumin, total protein, direct bilirubin and total bilirubin were obtained from Span Diagnostics Ltd. Surat, India.

Experimental Animal

Wistar albino rats (150-200g) were maintained in the animal house of Despanday labs, M.P.Nagar, Bhopal (M.P.) for experimental purpose. Then all the animals were acclimatized for seven days under standard husbandry conditions, i.e. room temperature of 25 ± 1^0 C; relative humidity 45-55% and a 12:12h light/ dark cycle. The animals had free access to standard rat pellet, with water supplied *ad libitum* under strict hygienic conditions. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol. All the protocols and the experiments were conducted in strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Experimental Study

DETERMINATIONS OF ACUTE ORAL TOXICITY (LD_{50})

The acute oral toxicity (AOT) of alcoholic and aqueous extract of aerial parts of *Chenopodium album* were determined by using female albino rats (Wistar strains) weighing between 180-220 g.The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD Guideline no. 425). Animals were administered with single dose of extracts dissolved in 2% w/v acacia and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. All the animals were also observed for long term toxicity (14 Days). The LD_{50} of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA.

EVALUATION OF HEPATOPROTECTIVE ACTIVITY

The alcoholic and aqueous extracts were evaluated for their hepatoprotective activity using paracetamol induced acute hepatic injury[19]. Wistar rats, weighing (180-220 g) were divided into 7 groups consisting of 6 animals in each group. Group 1 received distilled water for 7 days. Group 2 [Control (toxic)] were treated with vehicle (2 % acacia suspension, 1 ml/Kg, p.o.) for 7 days. Group 3 received silymarin (50 mg/Kg, p.o.) for 7 days. Group 4, 5 pretreated with alcoholic extract of aerial parts of *Chenopodium album* 200 mg and 400 mg/Kg respectively for 7 days. Group 6, 7 were pretreated with aqueous extract of aerial parts of *Chenopodium album* 200 and 400 mg/Kg respectively for 7 days. Food was withdrawn 16 hrs before paracetamol administration to enhance the acute liver damage in animals. Group 2, 3, 4, 5, 6, and 7 received a single dose of paracetamol (750 mg/Kg, i.p.) diluted with propylene glycol (12.5% solution) on 7^{th} day after 1 hrs of extract/test treatment and sacrificed 6 hrs after administration of paracetamol.

Blood samples were collected and serum was used for estimation of Asparate aminotransferase [AST], Alanine aminotransferase [ALT], alkaline phosphatase [ALP], albumin [ALB], total protein [TLP], total bilirubin [TBIL] and direct bilirubin [DBIL]. The liver was washed by normal saline, blotted with filter paper and weighed immediately[20].

Histopathological studies:

Liver was sliced and pieces were preserved in 10% formalin for proper fixation. These tissues were processed and embedded in paraffin wax. Section of 5- 6 microns in thickness were cut and stained with hematoxylin and eosin. All the sections of the tissues were examined under microscope for analyzing the altered architecture due to the liver tissue due to paracetamol challenge and improved liver architecture due to pretreatment with test extracts and standard drug[21] which was documented by photograph.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis was carried out using one-way ANOVA followed by Tukey Kramer's Post Hoc Test.

Results

Acute Oral Toxicity Study:

Different doses of alcoholic and aqueous extracts were screened for their oral toxicity. No mortality was recorded till 5000 mg/Kg with alcoholic and aqueous extracts, hence the extracts were found to be safe up to the dose levels of 5000 mg/Kg.

Paracetamol induced acute hepatic injury:

Liver weight and volume:
Administration of paracetamol has produced a significant increase in wet liver weight and volume. Rats pretreated with Silymarin (50 mg/Kg, p.o.), AQCA (400 mg/Kg, p.o.) and ALCA (400 mg/Kg, p.o.) showed significant decrease in liver weight and volume compared to the toxic control group [Table 1 and Fig 1].

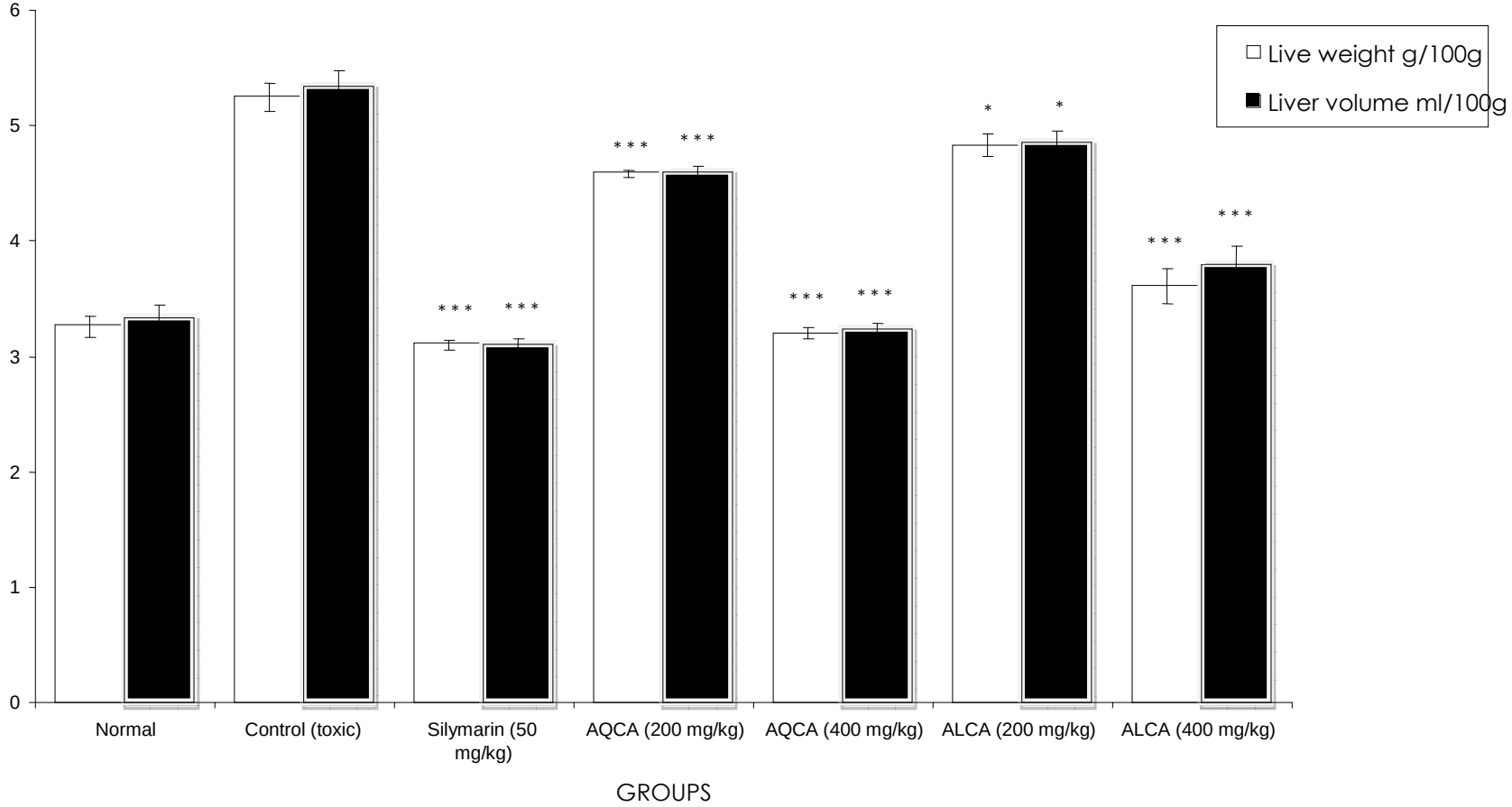
Table 1: Effect of Silymarin, AQCA and ALCA on total liver weight and volume in paracetamol induced liver damage in rats.

SNo.	Treatment	Mean liver weight (g/100g)	Mean liver volume (ml/100g)
1.	Distilled Water	3.27 ± 0.07	3.34 ± 0.10
2.	2 % w/v acacia + paracetamol (750 mg/Kg, i.p.)	5.26 ± 0 .10	5.34 ± 0.13
3.	Silymarin (50 mg/Kg, p.o.)+ paracetamol (750 mg/Kg, i.p.)	3.11 ± 0.03***	3.1 ± 0.05***
4.	AQCA (200 mg/Kg, p.o.)+ paracetamol (750 mg/Kg, i.p.)	4.59 ± 0.04***	4.6 ± 0.04***
5.	AQCA (400 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	3.2 ± 0.05***	3.23 ± 0.04***
6.	ALCA (200 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	4.83 ± 0.09*	4.86 ± 0.09*
7.	ALCA (400 mg/Kg, p.o.) +paracetamol (750 mg/Kg, i.p.)	3.61 ± 0.14***	3.8 ± 0.15***

Values are expressed as mean ± S.E.M; n = 6. * *P* <0.05, *** *P* <0.001 vs. control (Toxic), using one-way ANOVA followed by Tukey Kramer's Post Hoc test.

AQCA (alcoholic extract of *Chenopodium album*); ALCA (aqueous extract of *Chenopodium album*)

Fig 1: Effect of Silymarin, AQCA and ALCA on rat liver weight and wet liver volume in paracetamol induced liver damage in rats.



Serum Biochemical parameter:

* $P < 0.05$, *** $P < 0.001$ compared with control [toxic]

Paracetamol (750 mg/Kg, i.p.) administration resulted in significant elevated the biochemical parameters like AST, ALT, ALP, direct bilirubin and total bilirubin levels, while albumin and total protein were found to be decreased compared to normal group. Pretreatment with Silymarin, AQCA and ALCA significantly prevented the biochemical changes induced by paracetamol. The hepatoprotective effect offered by AQCA (400 mg/Kg, p.o.) was found to be significantly greater than ALCA (400 mg/Kg, p.o.) and standard (Silymarin 50 mg/Kg, p.o.) group. [Table 2 and Fig 2, 3, 4].

Table 2: Effect of Silymarin, AQCA and ALCA on different biochemical parameters in paracetamol induced hepatotoxicity in rats.

SNo.	Treatment	Serum biochemical parameters						
		AST IU/L	ALT IU/L	ALP IU/L	ALB g/dL	TLP g/dL	DBIL mg/dL	TBIL mg/dL
1.	Distilled Water	92.11 ±3.54	45.25 ±3.65	104.6 ±9.77	4.460 ± 0.29	15.95 ±0.95	0.23 ± 0.01	0.36 ±0.01
2.	2 %w/v acacia + paracetamol (750 mg/Kg, i.p.)	423.76 ±6.47	184.45 ±2.81	278.4 ±2.38	2.22 ± 0.29	7.077 ± 0.14	0.79 ± 0.05	1.06 ±0.04
3.	Silymarin (50 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	214.66 ± 2.14***	64.72 ± 2.55***	183.1 ± 10.93***	3.72 ± 0.17**	13.80 ± 0.38***	0.380 ± 0.020***	0.55 ±0.01***
4.	AQCA (200 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	305.73 ± 3.26***	83.87 ± 4.29***	218.93 ± 1.88**	2.53 ± 0.13	11.39 ± 0.40**	0.60 ± 0.01**	0.73 ±0.01***
5.	AQCA (400 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	219.4 ± 2.55***	54.55 ± 2.185***	161.43 ±14.36***	3.86 ± 0.08**	13.65 ± 0.17***	0.39 ± 0.03***	0.56 ±0.01***
6.	ALCA (200 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	386.21 ±10.37**	113.48 ± 5.77***	237.26 ±12.43	2.38 ± 0.32	9.83 ± 0.89	0.67 ± 0.09	0.84 ±0.03**
7.	ALCA (400 mg/Kg, p.o.) + paracetamol (750 mg/Kg, i.p.)	276.03 ± 7.97***	89.46 ± 4.014***	203.16 ± 7.12**	3.07 ± 0.07	11.39 ± 0.80**	0.50 ± 0.01***	0.64 ±0.01***

Values are expressed as mean ± S.E.M; n = 6. ** *P* <0.01, *** *P* <0.001 vs. control (Toxic), using one-way ANOVA followed by Tukey Kramer's Post Hoc test.

AQCA (alcoholic extract of *Chenopodium album*), ALCA (aqueous extract of *Chenopodium album)*, AST (asparate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), ALB (albumin), TLP (total protein), DBIL (direct bilirubin), TBIL (total bilirubin).

Fig 2: Effect of silymarin, AQCA and ALCA on serum AST, ALT, and ALP levels in paracetamol induced liver damage in rats.

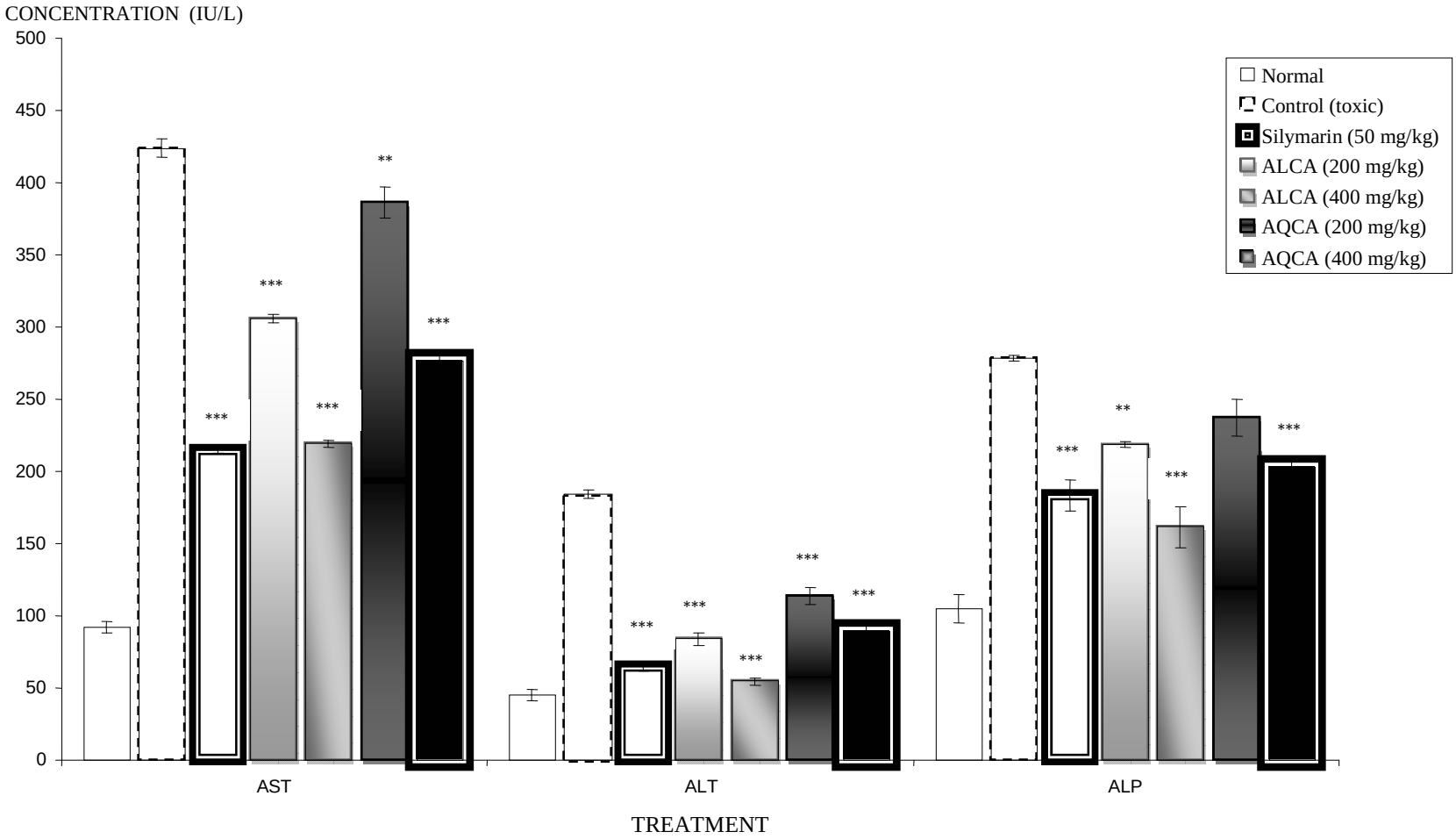
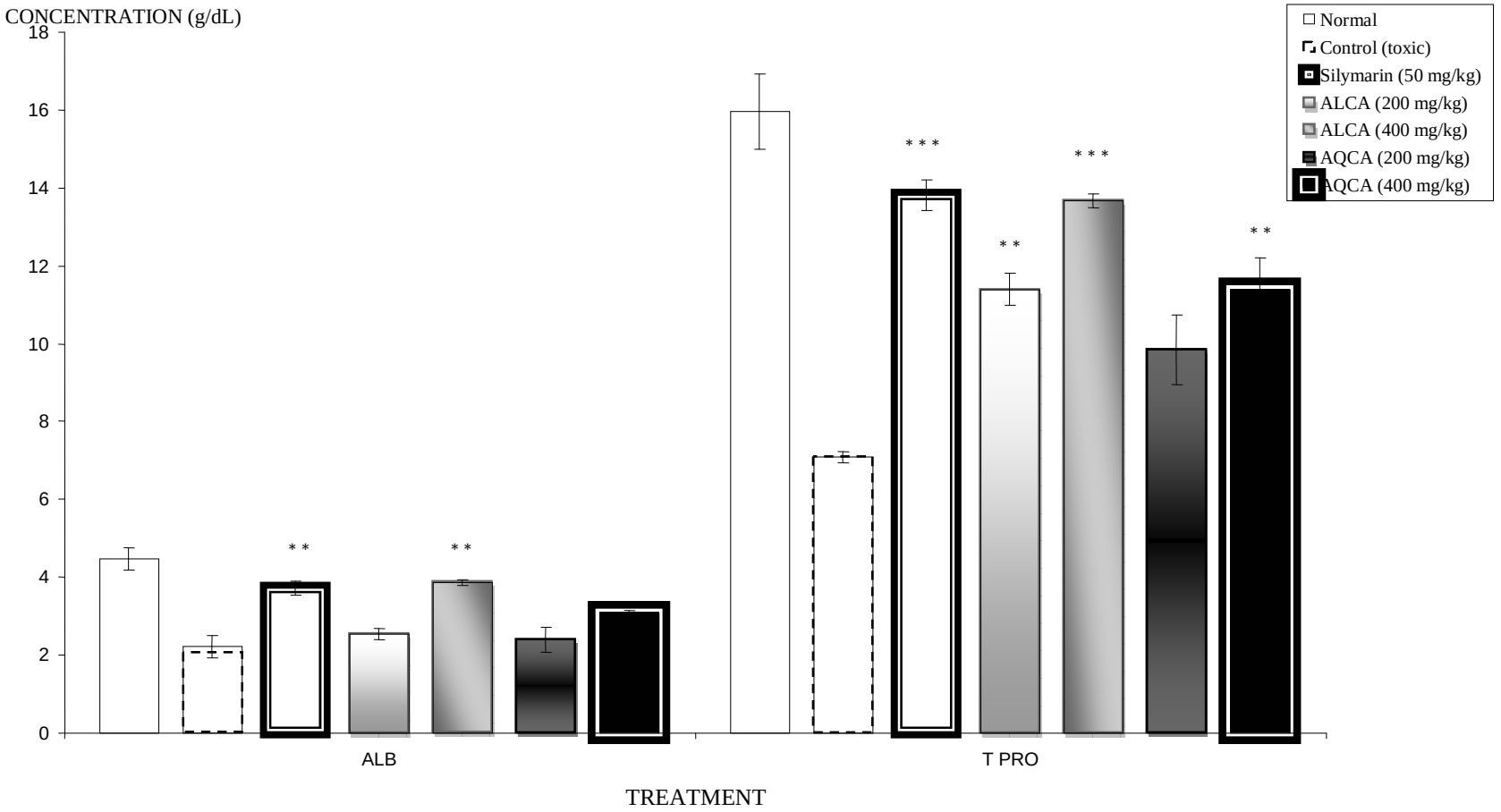
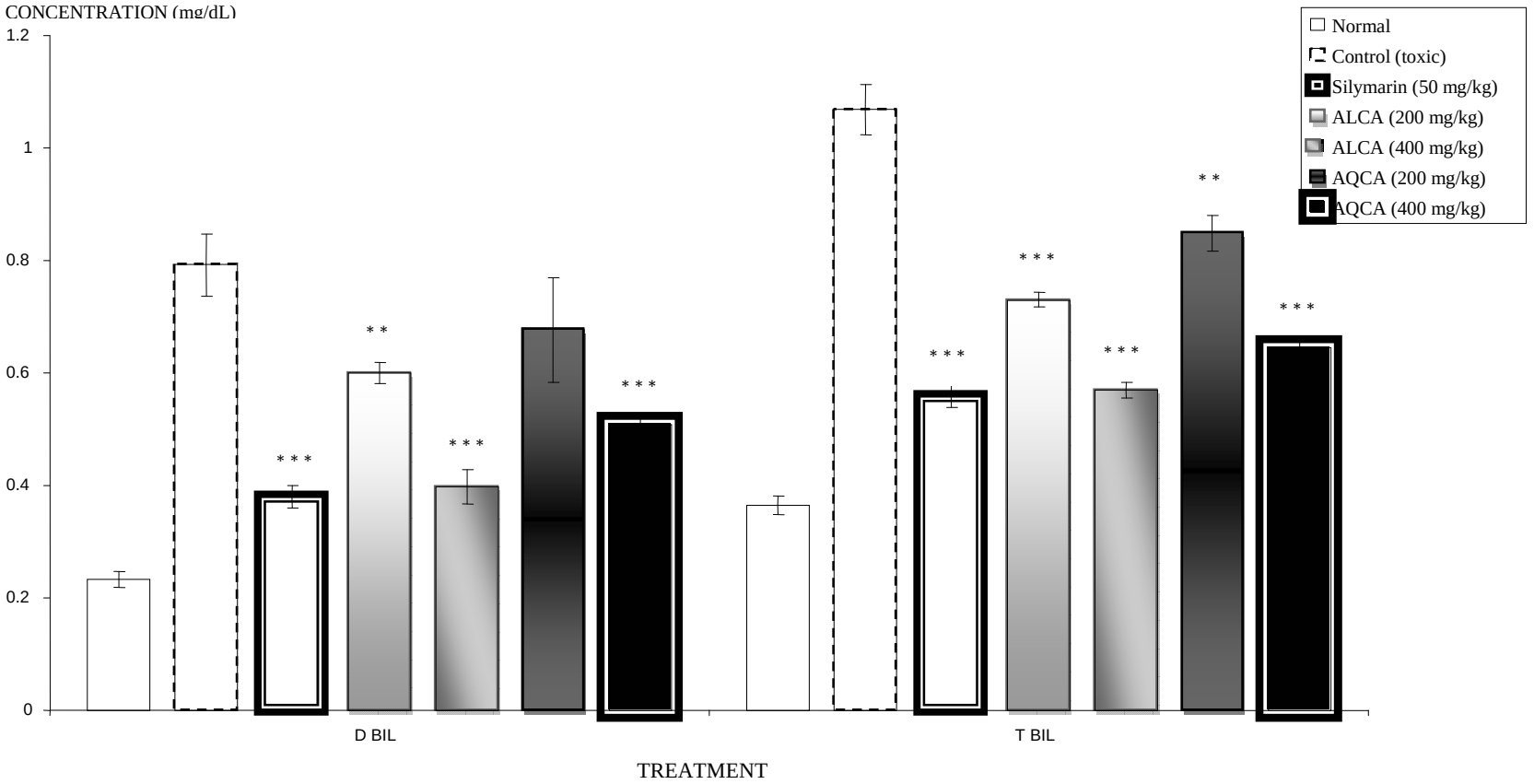


Fig 3: Effect of Silymarin, AQCA and AL s in paracetamol induced liver damage in rats.



** *P* < 0.01, *** *P* < 0.001 compared with control

Fig 4: Effect of Silymarin, AQCA and A racetamol induced liver damage in rats.



** *P* < 0.01, *** *P* < 0.001 compared with control

Histology:

In normal animals, liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleolus and central vein (Fig 5 A).

In paracetamol (750 mg/Kg, i.p.) treated animals, the sections showed moderate degree of liver damage, showing periportal and lobular inflammation and mild congestion (Fig 5B).

In AQCA (400 mg/Kg, p.o.) treated animals, liver section showing mild inflammation and congestion, overall picture resembles normal liver (Fig 5C).

In ALCA (400 mg/Kg, p.o.) treated animals, the liver sections showing moderate inflammation and congestion (Fig 5D).

In Silymarin (50 mg/Kg, p.o.) treated animals, the liver sections showing mild inflammation and moderate congestion (Fig 5E).

Fig 5: Sections stained with hematoxylin and eosin (400 X) displaying the liver sections of rats treated with normal, paracetamol, AQCA, ALCA and silymarin in acute paracetamol model.

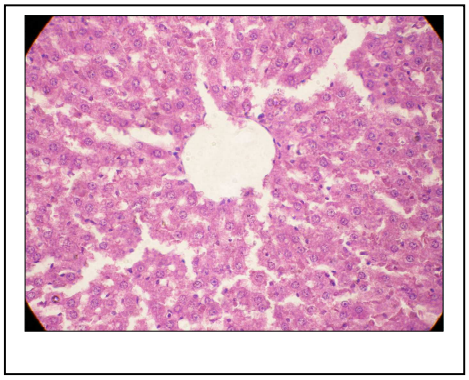


Fig: 5 [A] Normal histology of liver

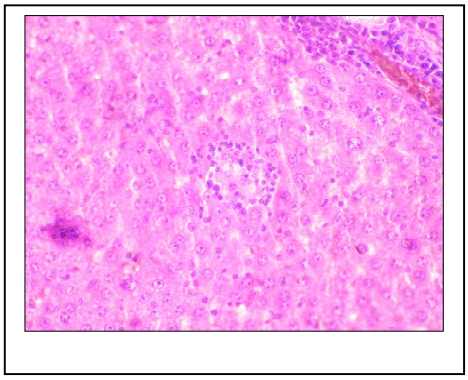


Fig: 5 [B] Control (toxic) group

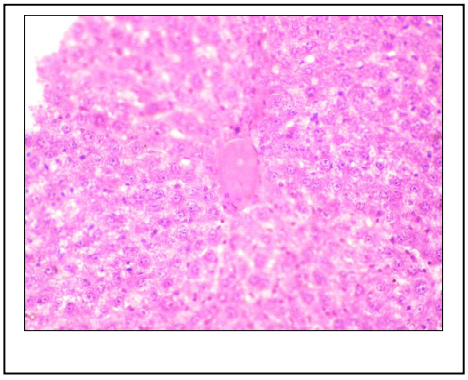


Fig: 5 [C] AQCA (400 mg/ Kg,p.o.)

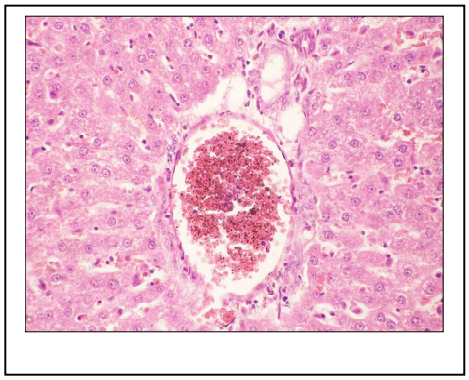


Fig: 5 [D] AQCA (400 mg/ Kg,p.o.)

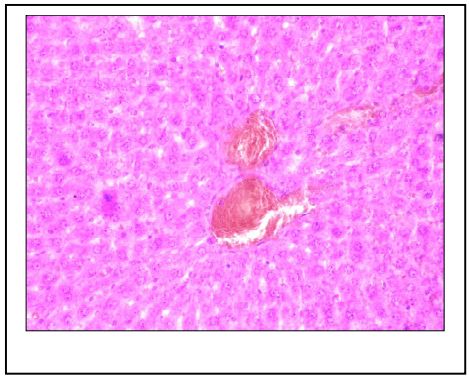


Fig: 5 [E] Silymarin (50 mg/Kg,p.o.)

Discussion

The liver can be injured by many chemicals and drugs. In the present study, Paracetamol was selected as a hepatotoxicant to induced liver damage. The primary objective of this study is to assess the hepatoprotective activity of *Chenopodium album* against chemically induced liver damage.

Paracetamol is well-known antipyretic and analgesic agent, which produces hepatotoxicity in higher doses[22]. Paracetamol hepatotoxicity is caused by the reaction metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion. Paracetamol toxicity is due to the formation of toxic metabolites when a part of it is metabolized by cytochrome P-450. Introduction of cytochrome[23] or depletion of hepatic glutathione is a prerequisite for paracetamol induced hepatotoxicity [24,25]. Normally, AST and ALP are present in high concentration in liver. Due to hepatocyte necrosis or abnormal membrane permeability, these enzymes are released from the cells and their levels in the blood increases. ALT is a sensitive indicator of acute liver damage and elevation of this enzyme in non hepatic diseases is unusual. ALT is more selectively a liver parenchymal enzyme than AST[26].

Assessment of liver function can be made by estimating the activities of serum ALT, AST, ALP and Bilirubin which are enzymes originally present higher concentration in cytoplasm. When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage[27].The elevated level of these entire marker enzymes observed in the group II paracetamol treated rats in this present study corresponded to the extensive liver damage induced by toxin. The reduced concentrations of ALT, AST and ALP as a result of plant extract administration observed during the present study might probably be due in part to the presence of flavonoids. Liver protective herbal drugs contain a variety of chemical constituents like phenols, coumarins, lignans, essential oil, monoterpenes, carotenoids, glycosides, flavonoids, organic acids, lipids, alkaloids and xanthenes[28].

Bilirubin is one of the most useful clinical clues to the severity of necrosis and its accumulation is a measure of binding, conjugation and excretory capacity of hepatocyte. Decrease in serum bilirubin after treatment with the extract in liver damage induced by paracetamol, indicated the effectiveness of the extract in normal functional status of the liver.

In experimental animals pretreated with *Chenopodium album* extracts (AQCA & ALCA) and silymarin, the total liver weight & volumes, AST, ALT, ALP, direct and total bilirubin levels were significantly lowered, while albumin and total protein significantly increased. Histopathological observation in experimental animals pretreated with AQCA, ALCA and silymarin showed similar to that of normal liver.

Thus result of present study clearly demonstrate that the various biochemical (serum AST, ALT, ALP, albumin, total protein, direct bilirubin and total protein levels), physical (liver weight & volume) and histopathological alterations produced by Paracetamol in the serum and tissue were reserved significantly by the pretreatment with extracts of *Chenopodium album* and in standard group.

From the results, the hepatoprotective activity of the extracts were in the order of AQCA (400 mg/ Kg, p.o.) > Silymarin (50 mg/Kg, p.o.) > ALCA (400 mg/Kg, p.o.) > AQCA (200 mg/Kg, p.o.) > ALCA (200 mg/Kg, p.o.).

Conclusion

The result of present study clearly demonstrate that the various biochemical, (serum AST, ALT, ALP, albumin, total protein, direct bilirubin and total protein levels), physical (liver weight & volume) and histopathological alterations produced by paracetamol in the serum and tissue were reserved significantly by the pretreatment of extracts of *Chenopodium album* and Silymarin. This study confirms its use as hepatoprotective as per the ethno pharmacological claims.

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