

Polygonum amplexicaule Extract: An Effective Herbal Cure to CCl₄ Induced Liver Damage *In Vivo*

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Abstract

Introduction: Liver damages are mainly caused by toxic chemicals, alcohol, infections and autoimmune disorders. *Polygonum amplexicaule* is a member of the genus *Polygonum* having high antioxidant contents and it has traditional medicinal attributes in treatment of many ailments particularly liver damage.

Objectives: Keeping in view the medicinal importance of *P. amplexicaule*, present study was constructed for *in-vivo* hepatoprotective activity of its rhizome extract in albino mice.

Methods: BALB/C albino mice were selected for experimental assays and sorted in nine groups (group i-iii control groups, group iv-ix herb treated groups) with six mice in each group for six days treatment. Blood samples from anesthetized mice (20% chloroform) were collected by carotid artery puncture allowed to coagulate at room temperature for 30 min and then centrifuged at 3000 rpm for 8-10 min. The serum separated was preserved at -20°C for subsequent analysis of biochemical parameters. Animals were sacrificed under diethyl ether anaesthesia at fasting state, liver tissues were quickly excised and were stored in fixative sera (10% formalin), for histology.

Results: Results revealed that CCl₄ had significantly reduced the body weight and increased the level of ALT, AST, ALP and plasma bilirubin compared to toxic control. The pre-treatment of CCl₄-administrated mice with extracts of *P. amplexicaule* at concentration of 200 mg/kg induced a profound decrease (62-94.4%) in the elevated activities of ALT, AST, ALP and plasma bilirubin level.

Conclusion: *P. amplexicaule* extracts have hepatoprotective activity and it can be used as herbal medicine for use in food, medicines and healthcare products to cure liver damages.

Keywords: *Polygonum amplexicaule*; Autoimmune disorders; Bilirubin

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Introduction

Carbon tetrachloride (CCl₄) is non-polar organic solvent with sweet smell like ether that can be detected at low level [1]. It is clear, heavy, non-inflammable liquid and used for induction of liver cirrhosis in animal model [2]. CCl₄ undergoes enzymatic activation in endoplasmic reticulum by cytochrome P-450 dependent mixed oxidase (CYP_{2E1}) and breaks down into highly toxic trichloromethyl CCl₃ and trichloromethyl peroxy (CCl₃O₂) free radicals [3]. These free radicals combine with cellular proteins

and lipids in aerobic conditions followed by chloromethylation and saturation, to induce lipid peroxidation [4,5].

Mammalian cells are equipped with both enzymatic and non-enzymatic antioxidant defences with different efficacies that protect cells against oxidative damage caused by wide range of toxins including CCl₄ [6]. To prevent the damage caused by free radicals, organisms have endogenous protective antioxidants. Toxic oxygen metabolites are detoxified by catalyzing reactions of important enzymatic antioxidants like SOD, CAT and GSH-Px [7].

Although there is tremendous advancement in the field of medicine but to stimulate liver functions still no effective drug is available in the market to cure liver and help hepatic cells to regenerate [8]. As compared to synthetic medicines, herbal medicines are more popular due to a number of reasons [9] such as they have no toxic effects and no side effects [10] are less cost effective [11] and much more easily handy [12,13]. So medicinal plants having important antioxidants and hepatoprotective activities was used in experimental animal model to cure liver damage. Due to its highest toxic effects CCl_4 is most widely used to induce toxicity in experimental models and used to study the hepatoprotective effects of synthetic and herbal drugs [2,4,5,8].

The genus *Polygonum* (Polygonaceae) having 150 species producing high contents of important secondary metabolites like flavonoids, phenolics, cardiac glycosides and terpenoids [14]. *Polygonum amplexicaule* (local name Masloon) having flowering season June to September is widely distributed in the North Pakistan having high antioxidant content. Locally *P. amplexicaule* is used for the treatment of liver damage, flue, fever, joint pain and gastrointestinal disorders and also other species to cure dysentery, heart problems, ulcer and menstruation [15].

Keeping in view the medicinal importance of *P. amplexicaule*, present study is constructed for determination of hepatoprotective activity of the medicinally important plant species rhizome extract on animal model.

Materials and Methods

Chemicals and reagents

All the chemicals and reagents used for these experiments are of analytical grade. Carbon tetrachloride, silymarin and olive oil were obtained from the drug control and traditional medicine division NIH, Islamabad. Diagnostic kits for ALT, AST, ALP and serum bilirubin estimation were also obtained from NIH, Islamabad.

Plant collection

Sample of *Polygonum amplexicaule* was collected from Murree hills and herbarium sheets were prepared, dried and then identified. Plant rhizome was used for this study. Whole work was done in National institute of health NIH, Islamabad.

Protocol for plant extraction

For preparation of plant extract, dried plant sample was grinded, sieved, Solution prepared, shaken and finally dried to get extract. A schematic procedure is given in flow chart extraction process (Figure 1).

Solution preparation

For extraction process solutions were made in polar solvents (distilled water, ethanol and methanol) in different ratios which are described in Table 1. For this purpose glassware like conical flask and cylinders were used. These glass wares were washed with distilled water and then sterilized. Sterilization process was done in an oven at 150°C for 15 min. This sterilized glassware's

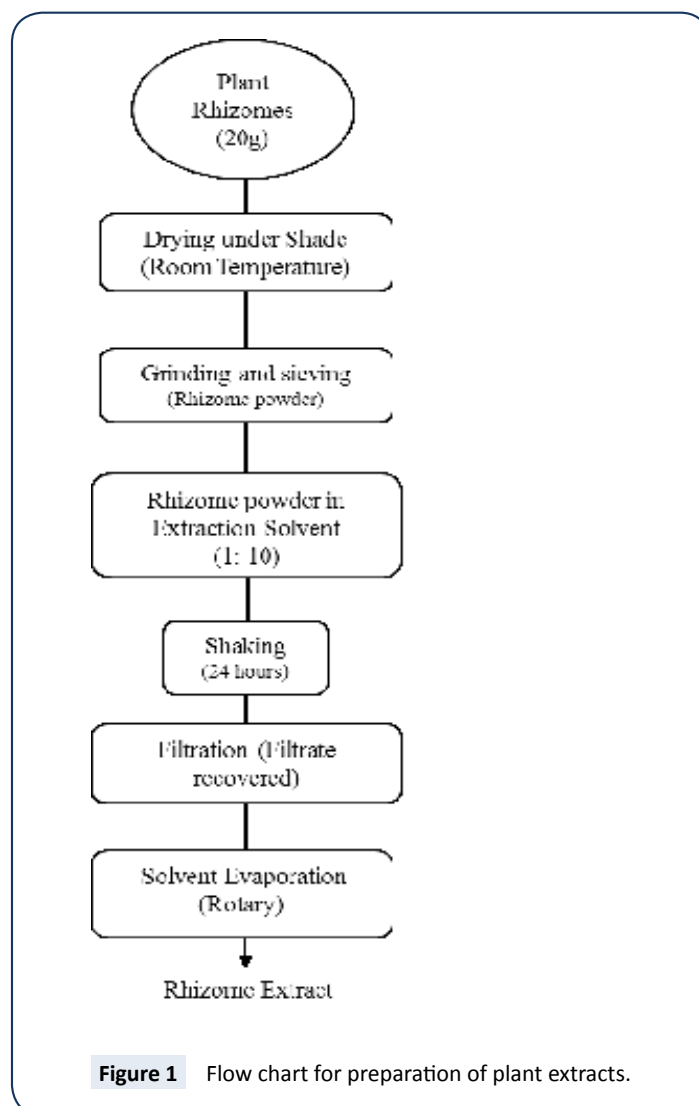


Figure 1 Flow chart for preparation of plant extracts.

Table 1 Amount of Plant material and volume of solvent for the preparation of extracts.

Plant	Solvent	Weight (g)	Volume (ml)
<i>Polygonum amplexicaule</i>	Methanol	20	200
<i>Polygonum amplexicaule</i>	Ethanol	20	200
<i>Polygonum amplexicaule</i>	Distilled Water	20	200

were used for preparation of solutions.

Respective amount of plant material were weighed with the help of electric weighing balance (Shimadzu) and volume of solvents like distilled water, ethanol and methanol was measured by using cylinders. Then plant materials were added into their respective type of solvent i.e., 20 g of *Polygonum amplexicaule* was added in 200 ml of ethanol, methanol and distilled water respectively. Conical flasks must be covered with rubber caps or aluminium foil [16]. Shaking of the solutions was done at 180 rpm at 36°C in Eyla shaking bath for 24 h. All samples were shaken 6 h daily and this process was completed in 4 days [16]. Solutions were filtered using Whatman filter paper of 42 cm pore size. Distilled water sample was filtered late as compared to methanolic and ethanolic samples [16].

Evaporation

After filtration, next step was to evaporate solvents from solution by using rotary vacuum evaporator. As the solution evaporates, it gets thick. Then that thick paste was transferred to falcon tubes. This thick paste must be in a dried form. For that purpose, these falcon tubes were placed in a water bath at 80°C. This process took 1-2 weeks. This dried form of plant extract was used for dose preparation [16].

Experimental animals

Healthy old Balb-C mice (28-40 g) of either sex were obtained from Animal house of NIH, Islamabad and were maintained on a standard laboratory diet. Mice were kept in standard poly-propylene cages at a 12-h cycle of light and dark. Room temperature at $24 \pm 5^\circ\text{C}$ and humidity maintained at 45-50%. All the chemicals used in this experiment were of the analytical grade, purchased from standard companies.

Toxicity studies

A study of acute toxicity was conducted according to the Organization for Economic Cooperation and Development (OECD) (OECD, 2001). A limited dose of 400 mg/kg of the aqueous, methanolic and ethanolic extracts of rhizome of *Polygonum amplexicaule* was used for the study.

Dose determination

Plant extract was dissolved in 0.9% normal saline solution. Calculations were as under:

For saline solution

Daily maximum dose consumption for each mouse was 100 μL . For six mice, 600 μL of saline solution was required. For six days, total dose administration was 3600 μL .

For 1 mice=100 μL ;

For 6 mice=600 μL ;

For 6 mice for 6 days=600 \times 6=3600 μL ;

Then, 3600 μL =3.6 ml.

Plant extract calculation

For 30 g of mice=400/1000 \times 30=12 mg;

For 6 mice=12 \times 6=72 mg;

For 6 days, 6 mice=72 \times 6=432 mg.

Total 432 mg of methanolic, ethanolic and aqueous extract was weighed independently and transferred into separate falcon tubes of 15 ml. Then 3.6 ml of saline solution was added to these falcon tubes with the help of Apple syringes. Methanolic extracts were reluctant to dissolve into saline as compared to aqueous extracts therefore; sonicator was used for efficient dissolving. Sonication was done at maximum level for 30 min.

Dose Administration for Toxicity Studies

Two groups each containing six mice were sorted in two cages. Each mouse was delivered with 0.1 ml of dose daily for consecutive six days.

Carbon Tetrachloride Induced Hepatotoxicity

BALB-C albino mice were selected for experimental assays and sorted in groups of six mice in each cage and each group received six days treatment of;

Group I: (Negative control) mice received normal food and water.

Group II: (Induction control 25% CCl_4 , 1 ml/kg) received normal saline daily.

Group III: Treated with standard drug silymarin (100 mg/kg b.w.).

Group IV-IX: Served as herb treated groups as below;

Group IV: Treated with *P. amplexicaule* (200 mg/kg b.w.) methanolic extract.

Group V: Treated with *P. amplexicaule* (300 mg/kg b.w.) methanolic extract.

Group VI: Treated with *P. amplexicaule* (200 mg/kg b.w.) ethanolic extract.

Group VII: Treated with *P. amplexicaule* (300 mg/kg b.w.) ethanolic extract.

Group VIII: Treated with *P. amplexicaule* (200 mg/kg b.w.) aqueous extract.

Group IX: Treated with *P. amplexicaule* (300 mg/kg b.w.) aqueous extract.

At the end of each treatment, mice were weighed and anesthetized with 20% chloroform. Blood samples from anesthetized mice were collected by carotid artery puncture allowed to coagulate at room temperature for half an hour. Blood samples were then centrifuged at 3000 rpm for 8-10 min. The serum separated was preserved at -20°C for subsequent analysis of biochemical parameters. Animals were sacrificed under diethyl ether anesthesia at fasting state, liver tissues were quickly excised and were stored in fixative sera (10% formalin), for histology.

Determination of body weight increase

Experimental animals were weighed before and after the onset of toxicity at the end of the trial and %age change in body weight were calculated for each animal using formula:

Change in weight (%)=[(final wt.-initial wt.)/initial wt.] \times 100

Biochemical Assays

Determination of Alanine-Aminotransferase (ALT) activity

ALT (E.C.:2.6.1.2) activity was determined according to methods described by Reitman and Frankel [17] using GPT-ALT LR reagent kit purchased from Merck, Germany.

Principle

Alanine+ α -ketoglutarate \xrightarrow{ALT} Pyruvate+Glutamate

Pyruvate formed reacts with 2,4-dinitrophenyl hydrazine to form pyruvate hydrazone complex which can be determined spectrophotometrically at 505 nm. Enzyme activity was obtained from standard curve by using absorbance of test samples.

Determination of Aspartate- Aminotransferase (AST) activity

AST (E.C.:2.6.1.1) activity on serum was determined as method described by Reitman and Frankel [17] having AST reagent kit obtained from Merck, Germany.

Principle

L-aspartate+ α -oxaloglutarate \xrightarrow{AST} oxaloacetate+L-glutamate

Oxaloacetate+NADH+H⁺ \xrightarrow{MDH} Malate+NAD⁺

The rate of absorbance changing at $\lambda=334$ nm is directly proportional to AST activity which was determined from standard curve.

Determination of Alkaline Phosphatase (ALP) activity

Activity of alkaline phosphatase was determined according to the method of Res [18] by using reagent kit purchased from Merck, Germany.

Principle

P-nitrophenylphosphate+H₂O \xrightarrow{ALP} p-nitrophenol+phosphate

The rate of p-nitrophenol formation is directly proportional to ALP activity which was determined from standard curve.

Determination of plasma bilirubin (Total)

Bilirubin level determination was done by the method described by Jendrassik and Grof [19] by using reagent kit purchased from Merck, Germany.

Principle

Bilirubin+diazotized Sulfanic acid \xrightarrow{HCL} Azobilirubin

Normal saline is used as an accelerator instead of caffeine, total bilirubin content couples with sulfanilic acid to form a red azobilirubin dye. The color intensity of the dye is proportional to the bilirubin concentration for direct bilirubin determination.

Statistical Analysis

The mean \pm SD was calculated for each parameter. Data for total variation was estimated by one way Analysis of Variance (ANOVA), followed by student's 't' test. P<0.001 was considered as statistically significant when compared to control group. The percentage of the protection is calculated as reported by Rawat et al. [20].

$$\text{Protection(\%)} = \frac{(\text{Mean value of } CCl_4\text{control} - \text{Mean value of test sample})}{(\text{Mean value of } CCl_4\text{control} - \text{Mean value of normal control})} \times 100$$

Results and Discussion

Hepatoprotective studies

This study was conducted to evaluate the hepatoprotective activity of *Polygonum amplexicaule* extract against carbon tetrachloride-induced toxicity in mice. In this study, hepatoprotective activity of aqueous, methanolic and ethanolic extracts of *P. amplexicaule* was examined at different doses.

Acute toxicity test of extracts of *Polygonum amplexicaule* rhizome

An experiment was performed to check toxicity level of plant extracts and results indicated that animals used for assessment of acute toxicity, normally gained their body weight as shown in **Table 2**. None of the mice died or showed any sign of toxicity at limited dose of 400 mg/kg in the first 48 h and no apparent evidence of toxicity was noted during the period of observation. This is evident from the results that any extract of *Polygonum amplexicaule* has no toxic or lethal effects up to dose of 400 mg/kg.

Results indicated a negative effect of carbon tetrachloride on body weight and positive body weight gain in those CCl₄-administered mice pretreated with standard drug (Silymarin) and different extracts of *P. amplexicaule* rhizome. Results are represented in **Table 3**.

CCl₄-administered mice exhibited a detectable decrease in weight as compared with normal mice (P<0.05). This decrease in the body weight may be due to alteration in nutrient absorption as

Table 2 Acute toxicity study of *Polygonum amplexicaule* rhizome.

S. No.	Plant extract	Body weight		
		Before experiment (g)	After experiment (g)	% age change
1	<i>Polygonum amplexicaule</i> methanolic extract	33.81 \pm 1.3	35.49 \pm 1.55	4.96 \pm 0.9
2	<i>Polygonum amplexicaule</i> ethanolic extract	40.63 \pm 1.03	42.67 \pm 1.05	4.99 \pm 0.2
3	<i>Polygonum amplexicaule</i> aqueous extract	37.18 \pm 1.46	38.58 \pm 1.83	3.77 \pm 0.05

Values expressed as means \pm S.D (n=6)

loss of food intake and metabolic utilization after pretreatment with CCl_4 as indicated by many publications [21,22].

The pretreatment of CCl_4 administered mice with standard silymarin and different concentrations of *P. amplexicaule* extracts exerted a significant raise in the body weight as compared to CCl_4 administered control group. $P < 0.01$ clearly revealed that results are highly significant ($12.96 \pm 1.13\%$) and ($16.3 \pm 1.12\%$) in case of methanolic and aqueous extracts of *P. amplexicaule* at concentration of 200 mg/kg b.w as compared to CCl_4 administered control ($-8.27 \pm 1.10\%$).

Significant increase in the body weight might be due to high flavonoid and phenolic constituent which may improve the nutritional and healthy states leading to an enhanced body weight gain. The high flavonoid diet is reported for decrease in weight caused by CCl_4 exposure Krishna et al. [21] and Singh et al. [23].

Effect of Plant Extract on Serum Enzyme Activities and Bilirubin Total Proteins

In the present study, toxic effect of CCl_4 cause extensive damage to the liver, reflected by the elevation of serum ALT, AST, ALP as well as rise in plasma bilirubin levels.

ALT activity

ALT is the most reliable enzyme used to indicate hepatic damage to hepatocytes [24]. ALT is more selectively a liver parenchymal enzyme than AST. Data are showing the effect of methanolic, ethanolic and aqueous extracts of *P. amplexicaule* on serum ALT level in CCl_4 -administered mice were represented in **Tables 4** and **5**.

CCl_4 -administered mice exhibited a very highly significant increase ($P < 0.001$) of ALT level (264 ± 5.91) as compared to normal control

Table 3 Effects of *Polygonum amplexicaule* on body weight gain in CCl_4 administered mice.

Groups	Body Weight (g)		
	Before Experiment	After Experiment	Change in Weigh (%)t
Normal control	20.33 ± 0.25	24.43 ± 0.28	20.16 ± 2.40
Solvent control Normal saline (10 ml/kg)	20.14 ± 0.52	22.72 ± 0.24	12.33 ± 1.82
Induction control (25% CCl_4 , 1 ml/kg)	20.55 ± 0.23*	18.98 ± 0.50*	-8.27 ± 1.10*
Drug control (Silymarin 100 mg/kg)+ CCl_4	23.51 ± 0.23 ^λ	26.07 ± 0.16 ^λ	10.88 ± 1.12 ^λ
<i>Polygonum amplexicaule</i> methanolic extract (200 mg/kg)+ CCl_4	25.29 ± 0.27 ^α	28.57 ± 0.31 ^α	12.96 ± 1.13 ^α
<i>Polygonum amplexicaule</i> methanolic extract (300 mg/kg)+ CCl_4	20.41 ± 0.23	21.84 ± 0.21	7.00 ± 0.13
<i>Polygonum amplexicaule</i> ethanolic extract (200 mg/kg)+ CCl_4	25.72 ± 0.61 ^α	27.80 ± 0.29 ^α	8.08 ± 0.59 ^α
<i>Polygonum amplexicaule</i> ethanolic extract (300 mg/kg)+ CCl_4	24.99 ± 0.30	26.66 ± 0.41	6.68 ± 0.12
<i>Polygonum amplexicaule</i> aqueous extract (200 mg/kg)+ CCl_4	21.91 ± 0.92 ^{βA}	25.49 ± 1.08 ^{βA}	16.3 ± 1.32 ^{βA}
<i>Polygonum amplexicaule</i> aqueous extract (300 mg/kg)+ CCl_4	28.11 ± 0.91	29.89 ± 0.81	6.37 ± 0.50

Data are expressed as mean ± S.D (n=6)

Values differ significantly compared with normal control: * $P < 0.05$

Values significantly different compared to CCl_4 -administered control: ^α $P < 0.05$, ^λ $P < 0.01$

Values significant varied comparable to standard drug: ^β $P < 0.001$

Table 4 Effect of *Polygonum amplexicaule* extracts on serum enzymes and plasma total bilirubin.

Groups	Treatment	Biological parameters			
		ALP (U/L)	AST (U/L)	ALT (U/L)	Plasma Total Bilirubin (mg/dl)
I	Normal control	130.46 ± 6.76	48.36 ± 6.74	39.472 ± 4.24	0.143 ± 0.06
II	Induction control (25% CCl_4 , 1 ml/kg)	640.22 ± 8.40*	169.36 ± 8.80**	264.87 ± 5.91*	1.24* ± 0.12
III	Control (Silymarin 100 mg/kg)+ CCl_4	281.45 ± 7.60 ^λ	50.665 ± 6.72 ^α	178.022 ± 1.25 ^λ	0.242 ^α ± 0.07
IV	<i>Polygonum amplexicaule</i> methanolic extract (200 mg/kg)+ CCl_4	295.76 ± 4.48	54.52 ± 6.41	125.26 ± 4.58	0.172 ± 0.06
V	<i>Polygonum amplexicaule</i> methanolic extract (300 mg/kg)+ CCl_4	273.29 ± 5.80	57.55 ± 6.01	63.26 ± 2.96	0.153 ± 0.02 ^{αβ}
VI	<i>Polygonum amplexicaule</i> aqueous extract (200 mg/kg)+ CCl_4	221.64 ± 2.56	82.402 ± 4.40	90.73 ± 5.31	0.194 ± 0.02 ^α
VII	<i>Polygonum amplexicaule</i> aqueous extract (300 mg/kg)+ CCl_4	264.42 ± 8.22	62.28 ± 8.44	73.37 ± 6.10	0.153 ± 0.02
VIII	<i>Polygonum amplexicaule</i> ethanolic extract (200 mg/kg)+ CCl_4	209.39 ± 10.1 ^{αβ}	71.446 ± 1.62	65.141 ± 2.2	0.273 ± 0.04 ^λ
IX	<i>Polygonum amplexicaule</i> ethanolic extract (300 mg/kg)+ CCl_4	234.16 ± 7.81	57.34 ± 3.89	82.93 ± 7.41	0.163 ± 0.01

Values expressed as means ± S.D (n=6)

Values significantly varied comparable to normal control: * $P < 0.001$, ** $P < 0.01$

Values significantly varied comparable to CCl_4 -administered control: ^α $P < 0.01$, ^λ $P < 0.05$

Values significantly varied comparable to Drug control: ^β $P < 0.05$

Table 5 Percent of protection of various biochemical parameters, as compared to induction control group (CCl₄ group).

Treatment	ALP (U/L)	AST (U/L)	ALT (U/L)	Plasma Total Bilirubin (mg/dl)
Control (Silymarin 100mg/kg)+CCl ₄	70.40%	98%	82.9	88%
<i>Polygonum amplexicaule</i> (P.A) methanolic extract (200mg/kg)+CCl ₄	67.60%	95%	62%	93.85%
<i>Polygonum amplexicaule</i> (P.A) methanolic extract (300mg/kg)+CCl ₄	72%	92%	90%	96%
<i>Polygonum amplexicaule</i> (P.A) aqueous extract (200mg/kg)+CCl ₄	82.10%	71.80%	77.02%	92%
<i>Polygonum amplexicaule</i> (P.A) aqueous extract (300mg/kg)+CCl ₄	73.80%	88.40%	85%	96%
<i>Polygonum amplexicaule</i> (P.A) ethanolic extract (200mg/kg)+CCl ₄	84.50%	81%	89%	85%
<i>Polygonum amplexicaule</i> (P.A) ethanolic extract (300mg/kg)+CCl ₄	80%	92%	81%	94.40%

(39.472 ± 4.24). Elevation of serum ALT clearly indicated the toxic effect of CCl₄ cause extensive damage to the liver because it is localized in cytoplasm and released after cellular damage [25].

The pretreatment of CCl₄-administered mice with rhizome extracts of plant produced a very significant decrease (P<0.01) in case of ethanolic extracts (65.141 ± 2.2) at concentration of 200 mg/kg b.w and decrease (P<0.05) in case of aqueous extract 200 mg/kg (90.73 ± 5.31), aqueous extract 300 mg/kg (73.37 ± 6.10) and ethanolic extracts (82.93 ± 7.41) at concentration of 300 mg/kg b.w as compared to CCl₄ administered control group. These extracts when compared with standard drug (78.02 ± 1.25) revealed that aqueous extract and ethanolic extracts are significantly varied (P<0.05) to drug. Several studies have been reported on hepatoprotective activity of medicinal plants. Among these, results of ALT reported by Pendit et al. [26] were quite similar to our present studies. ALT level of pretreated CCl₄-administered rats with *Adhatoda vasica* was reported as 49.0 ± 5.9 as compared to CCl₄-administered control group (Figures 2 and 3).

AST activity

AST presents two isozymes, one is present in mitochondria while the other one in cytoplasm [27]. Data are showing the significant rise in AST level in toxic control while highly significant decrease in groups treated with different concentrations of extracts.

It has been found highly significant rise (P<0.05) in serum AST level in CCl₄-administered control group (169.362 ± 2.17) as compared to normal control taken as negative control (48.36 ± 7.79).

The pretreated mice with plant extracts have significantly lowered (P<0.01) AST level. Methanolic, ethanolic, aqueous extracts of plant having 300 mg/kg dose and methanolic extracts of 200 mg/kg showed same effect on lowering AST level up to 57.55 ± 6.01, 57.34 ± 3.89, 62.28 ± 8.44 and 54.52 ± 6.41 respectively as compared to CCl₄-administered toxic control having AST value of 169.36 ± 8.8. Ethanolic and aqueous extracts were further showed significant variation (P<0.05) when compared with standard drug control having AST of 50.665 ± 6.7. Pendit et al. [26] have reported AST level in case of pretreated rat with plant extract but with dose of 200 mg/kg b.w which is close to our study.

ALP activity

ALP is present in hepatocytes plasma membrane and elevation in ALP level is related directly to the damage in liver cell membrane



Figure 2 Mice sorted for assessment of hepatoprotective activity.

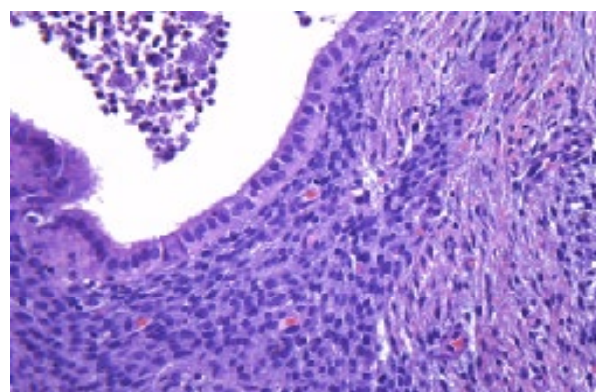


Figure 3 Dose administration to mice.

[28,29]. Elevated level of ALP in toxic condition and reduced level in case of treatment with drug and extracts of *P. amplexicaule* is shown.

Results indicated in **Table 4** show significant increase of ALP in CCl₄-administered control group as compared to normal non-toxic group. Normal value of ALP in mice were found to be 130.46 ± 6.76 which rose significantly (P<0.001) to respective value of 640.22 ± 8.40 after administration of toxic dose of CCl₄ (1 ml/kg).

Pretreatment of CCl₄ administered mice with different extracts of *P. amplexicaule* returned the values to normal indicates the preventive effects of plant. Among these extracts, ethanolic and aqueous extracts of 200 mg/kg returned the serum ALP level to 209.39 ± 10.1 and 221.64 ± 2.56 respectively which were

significantly lower ($P < 0.01$) than the value of toxic control and non-significantly ($P > 0.05$) close to normal values. The values also significantly ($P < 0.05$) varied when compared with drug control (281.45 ± 7.60) compared to the lowering level of ALP reported by Pendit et al. [26].

Plasma bilirubin level

Serum bilirubin level estimation is another one of the most important tests used for hepatic diseases diagnosis which measures the binding, conjugation and excreting capacity of hepatocytes proportional to erythrocytes degradation rate [23]. The present data show the bilirubin level in normal, diseased and mice pretreated with different extracts of plant sample.

Results shown in **Table 4** indicated that in case of CCl_4 -administered group there is a highly significant rise ($P < 0.001$) in the level of plasma bilirubin (1.19 ± 0.12) as compared to non-treated mice (0.246 ± 0.06). This increased level of serum bilirubin is due to excessive production of bilirubin because of

breakdown of RBCs and the inability of animal to excrete bilirubin due to liver damage [29]. The increase of plasma bilirubin levels by CCl_4 further confirmed that CCl_4 is toxic for liver which agrees with Ahsan et al. [16]; Vogel [30]; Samudram et al. [31].

Among these treatments results significantly lowered ($P < 0.01$) in case of ethanolic and aqueous extracts of 200 mg/kg having respective bilirubin levels of 0.273 ± 0.04 and 300 mg/kg 0.194 ± 0.02 and methanolic extract of 300 mg/kg 0.153 ± 0.03 as compared to CCl_4 toxic control. Further comparison of these extracts with standard drug revealed that among these methanolic extracts is significantly varied ($P < 0.05$) which is closely related with the results as described by Krishna et al. [21].

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