

# Polygonum persicaria (linn) aqueous extract has hepatoprotective efficacy against carbon tetrachloride toxicity in rats.

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## Abstract

Therapeutic plant preparations may contain critical levels of poisonous substance constituents to possibly make genuine damage to animals as well as people. Accordingly, toxicity examines are significant to possibly make toxic impacts of plant derived products. Natural medications from therapeutic plants are seen as a powerful and safe discretionary treatment for liver injury. The acute toxicity study demonstrated that the aqueous extract of *Polygonum Persicaria* is harmless even at the highest dosage of 2000 mg/kg in albino Wistar rats. There were no behavioural or physiological changes and gross pathological irregularities watched. The toxicity examination of aqueous extract of *Polygonum Persicaria* at 5, 50, 300, and 2000 mg/ kg were led in albino Wistar rats. The current examination was directed to evaluate the hepatoprotective association of aqueous extract of basic roots of *Polygonum Persicaria* at an oral dosage of 200 and 400 mg/kg in wistar rats. The investigations were coordinated with the prompting specialist CCl<sub>4</sub> (1.5 ml/kg i.p). Silymarin (100) mg/kg p.o) was used as a kind of perspective cure in particular models. The effect was tested by enzymatic level assessments and histopathological examinations. *Polygonum Persicaria* aqueous extract has demonstrated significant hepatoprotection against CCl<sub>4</sub> induced hepatotoxicity study models in wistar rats. This has been illustrated by a pronounced reduction in serum marker proteins. The protecting ability of *Polygonum Persicaria* against hepatoprotective stimuli may be due to the phenolic or flavonoid compounds that we have found through phytochemical analysis. The hepatoprotective design of the extract was also affirmed by histopathological tests.

**Keywords:** Carbon tetrachloride, acute toxicity, hepatoprotective, *Polygonum Persicaria*, Silymarin, Histopathology.

## I. INTRODUCTION:

Liver is the fundamental organ of digestion and discharge. As a consequence of its central structure in the body, it is ceaselessly and differently introduced to xenobiotics. Hepatic injury is related through bending of metabolic capacities, so liver disease remains one of the real medical challenges [Wolf P L, 1999]. Medication incited liver damage is a significant medical issue that challenges social insurance experts as well as the pharmaceutical business and administrative offices. Medication prompted liver damage remains answerable for 5% of all emergency clinic affirmations besides half of totally liver disappointment [Friedman et al., 2003]. In excess of 900 medications have been worried in making liver damage and it is the most natural explanation behind a medication to be stopped from the marketplace. Modern prescriptions have little to bring for mitigation of hepatic sicknesses and it is fundamentally the plant-based arrangements which are used for their treatment of the liver problem.

Carbon tetrachloride is the best portrayed representation of xenobiotic-prompted hepatotoxicity and is regularly utilized for the screening of hepatoprotective impacts of drugs as well as normal products. Carbon tetrachloride (CCl<sub>4</sub>) liver damage relies on the metabolism of trichloromethyl radicals (CCl<sub>3</sub>• or probably CCl<sub>3</sub>OO•) under cytochrome P-450 activity [Weber et al., 2003; Manibusan et al., 2007]. The trichloromethyl radical responds to different essential biomolecules, such as unsaturated fats, proteins, lipids, nucleic acids, and amino acids, inducing lipid peroxidation and breaking down the mixture and division of DNA. Therefore, producing cellular breakdown revealed by deviations in haematological and biochemical parameters [Girish et al., 2009]. Consequently, the balance between the development of reactive oxygen species (ROS) and the antioxidant defense mechanism is disrupted by oxidative stress by undermining cellular potential due to CCl<sub>4</sub>-prompted liver injuries. In spite of the extensive progressions in medication, drugs utilized for liver care have numerous side effects which compound the condition. It is also important to find healthy alternatives to replace chemical medicines from traditional medicines by the use of many investigational models [Muriel and Rivera., 2008; Patwardhan, et al., 2004]. The preventive properties of natural antioxidants in natural medicines and disengaged bioactive constituents, which are known to be the best and safer hepatotoxicity therapies, have been given greater consideration in this unique situation [Grajales and Muriel., 2015].

The plant *Polygonum persicaria* (*P.P*) Linn. (Polygonaceae) is utilized in the Siddha method of medication. The plant possess a broad spectrum of antibiotic, antibacterial, and

anticancer activity. The extract of the aqueous roots of *Polygonum persicaria* has demonstrated anti-inflammatory, antimicrobial and anticancer efficacy in preliminary pharmacological investigations of the herb. The new essential fundamental (roots) of *Polygonum persicaria* have against cancer-causing activity [Duwiejua M et al., 1999]. *Polygonum persicaria* has been represented to contain a couple of dynamic parts including taxifolin, myricitrin, luteolin, protocatechuic acid, arctiin, lappaol B, naringenin, etc., that have been identified in various phytochemical tests [Li YJ et al., 2005; Zheng S et al., 1997]. Some significant pharmacological impacts, for example, cardioprotective, antidiabetic, antioxidant and osteoblastic were seen in the plant extract [Wei Y et al., 2009; Nigam V et al., 2013; Liao SG et al., 2013; Xiang MX et al., 2011]. In this study to explore the hepatoprotective impact of *Polygonum persicaria*.

## II. MATERIALS AND METHODS:

### Plant material and preparation of the extracts.

The new plant material *Polygonum persicaria* was gathered from Lethpora, Pulwama Kashmir near to the Jhelum River and was recognized by undersigned at centre for biodiversity and Taxonomy, Department of Botany, University of Kashmir. With voucher specimen Herbarium No. **2925-(KASH)**. The plant material was washed with water, cut into pieces, and dried at room temperature. The dried plant material were then crushed into coarse powder in a crushing machine. The plant sample of 500g was extracted in purified water for a period of 3 days. Solvent from sample was filtered, crushed off and vanished off under condensed force in a rotary evaporator to acquire crude extract. A voucher model was kept in our lab for future reference.

### Phytochemical evaluation.

The preliminary phytochemical screening in the aqueous extract of *Polygonum persicaria* for constituents such as steroid, alkaloid, tannin, flavonoid and glycoside was carried out according to the method described by Harborne (1984).

### Acute toxicity studies.

According to the OECD-423 guidelines, an acute oral toxicity study has been carried out. Medications were administered orally in groups of rats in portions of 5, 50, 300, 2000 mg/kg body weight (n = 3) and the percentage mortality was reported over a 24-hour period. Rats

were tracked for gross behavioural changes within the initial 1 hour of drug organization, as depicted by Irwin S et al., 1968. In the event that death was observed in two out of three animals, the dosage given was allocated as a lethal dose at that time. In the event that death was detected in one animal, the same dosage was replicated at that stage to validate the poisonous dose. As mortality was not observed, for additional higher dosages such as 50, 300 and 2000mg/kg body weight, the process was replicated.

### **Experimental Animals.**

Studies were performed using adult male albino rats ( $130\pm 10$ g/12-16 weeks) selected from departmental colonies and housed in well-ventilated stainless steel cages at room temperature ( $24\pm 2^\circ\text{C}$ ) under natural light and dark plan in hygienic condition and were fed on a normal laboratory diet. Food and water were given ad libitum. Experiments were carried out at the Pinnacle Biomedical Research Institute (PBRI) in Bhopal, India (Reg. No.1824/PO/Ere/S/15/CPCSEA) following approval by the Institute's Animal Ethics Committee (IAEC) in compliance with OECD guidelines. Animals have been treated and considered in compliance with the rules proposed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

### **Experimental design for hepatoprotective activity.**

Animals were divided into five groups of six rats each. Group –I served as normal received only the vehicle (5% gum acacia; 1ml/kg; p.o), group –II as a toxic control, received only  $\text{CCl}_4$  (1.5 ml/kg) (1:1 of  $\text{CCl}_4$  with olive oil i.p.), group-III act as a standard received silymarin at an oral dose of 100 mg/kg, and group –IV–V received the aqueous extract of polygonum persicaria at an oral dose of 200 & 400 mg/kg, as a fine suspension of 5% aqueous gum acacia. The treatment were continued for 14 days, once daily. On the day of 14 for groups III-V, 30 min post-dose of extract administration animals received  $\text{CCl}_4$  at the dose of 1.5 ml/kg (1:1 of  $\text{CCl}_4$  with olive oil i.p).

### **Collection of Samples.**

On completion of the time of experimentation. Blood samples were obtained from the orbital plexus at 24 h after the last  $\text{CCl}_4$  injection, under light ether anaesthesia, into non-heparinized capillary tubes. The serum was isolated at 3000 rpm by centrifugation for 5 minutes and processed before examination at  $-20^\circ\text{C}$ . The liver was detached, washed with saline, weighed and then 10% (w/v) homogenate of the liver was prepared in ice cold saline.

Thiopental sodium (50mg/Kg) were used as anaesthetized for animals. The blood was extracted and EDTA was added as an anticoagulant. The serum was separated by centrifugation and used for various biochemical studies. A section of the liver was fixed in 10% formalin for histopathological examinations.

### Histopathological studies.

The liver tissue was fixed in 10% normal saline for 72h after which the tissues were sliced to a thickness of 2.1mm each. These were dehydrated using alcohol of graded concentration. They were further treated with paraffin wax and cast in to blocks. Segments of the tissues were sliced on a microtome to 5 $\mu$ m. These were subsequently attached to a slide and dried. The samples slides were viewed on a photographic microscope to find out histological changes.

### Statistical analysis.

All values have been expressed as mean  $\pm$  SD (in each category n=6). To assess the importance of the biochemical data of the various groups, one-way ANOVA was applied. The value is set at  $p < 0.001$ .

## III. RESULTS:

The results of phytochemical screening are described in **Table 1**.

**Table 1:** Results of phytochemical screening of Polygonum persicaria aqueous sample.

Phytochemical compound	Presence/absence
Carbohydrate	+ve
Cholesterol	+ve
Steroid	+ve
Alkaloid	+ve
Tannin	+ve
Saponin	+ve
Phenol	+ve
Cardiac glycoside	+ve

+ve  $\implies$  presence; -ve  $\implies$  absence.

### Acute toxicity studies.

No poisonous signs or mortality were caused by aqueous extracts of *Polygonum persicaria* up to the dose level of 2000 mg/kg body weight in rats, so the extract was harmless and non-toxic for further pharmacological studies.

### Hepatoprotective activity.

The animals treated with toxic dosages of CCl<sub>4</sub> had especially raised estimations of the serum ALT, AST, ALP & total bilirubin contrasted with normal animals, showing intense hepatocellular damage (**Table-1**). Serum enzymes values in the animals pre-treated with the aqueous extract of *Polygonum persicaria* (200 & 400 mg/kg; p.o) were essentially lower than those of harmful control values and aside from ALT, AST, ALP & total bilirubin serum enzymes qualities in treated animals were like the normal values. The effects of *Polygonum persicaria* aqueous extract were practically equivalent to that of standard silymarin activity.

**Table-2. Pre-treatment impact of *Polygonum persicaria* aqueous extract on CCl<sub>4</sub>-induced rats.**

Groups	Treatment	ALT* (U/L)	AST* (U/L)	ALP* (U/L)	TB* (mg/dl)
I	Control-Normal	54.36±5.69	142.08±11.10	139.68±7.42	0.51±0.10
II	CCl <sub>4</sub> -treated	209.24±10.12	287.69±60.12	345.26±74.08	2.04±0.25
III	SLY* 100mg/kg+CCl <sub>4</sub>	56.41±14.98	150.58±15.86	151.15±12.05	0.55±0.08
IV	P.P* 200mg/kg +CCl <sub>4</sub>	120.20±49.12	222.07±16.71	254.40±37.61	1.51±0.09
V	P.P* 400mg/kg +CCl <sub>4</sub>	83.06±10.77	184.65±21.08	184.45±26.37	0.81±0.07

P.P\* -*Polygonum persicaria*, SLY\*- Silymarin, ALT\*-alanine transaminase, AST\*-aspartate aminotransferase, ALP\* - alkaline phosphatase, TB\*-total bilirubin.

### Histopathological studies.

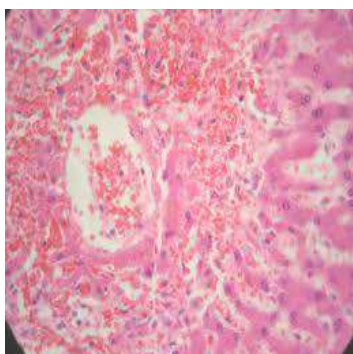
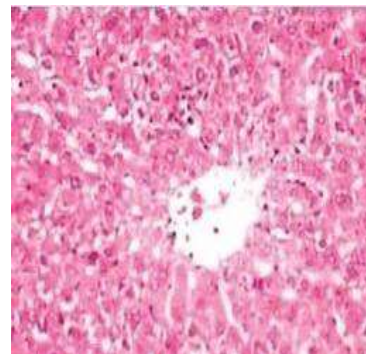
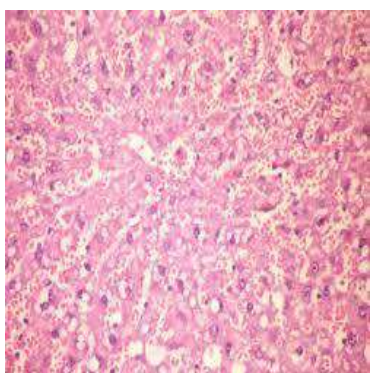
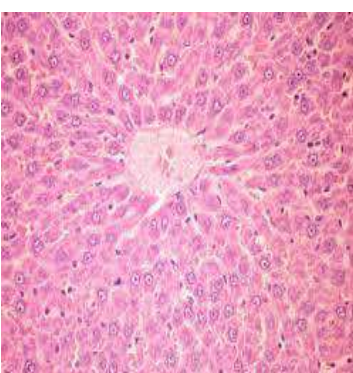
In order to support the serum analysis findings and the hepatoprotective effects of the drugs, histopathological analyses of the liver portion of rats were carried out. The findings obtained from serum catalyst studies is supported by histopathological observations. There have been reported histopathological modifications in the liver caused by CCl<sub>4</sub>. Pre-treatment with silymarin and extracts reversed the effect of CCl<sub>4</sub>.

Histopathological investigation of normal group (Fig. A) animals displayed a regular design of different cells, central vein clear and sinusoidal spaces. Interestingly, the CCl<sub>4</sub> bunch indicated liver wounds, for example, moderate to serious corruption around the focal

vein, neutrophils penetration, swelling degeneration & loss of cell boundaries (Fig. B). Rats treated with silymarin displayed nearly standard uniform sinusoidal architecture (Fig. C). Pre-treatment with aqueous extract of *Polygonum persicaria* (200 mg/kg (Fig. D) revealed a relatively normal pattern with a slight degree of necrosis and inflammatory cells infiltration compared to CCl<sub>4</sub> group, & and pre-treatment of *Polygonum persicaria* at 400 mg/kg (Fig. E) almost shows normal structure which is almost similar with silymarin.



Fig. A (Normal group)

Fig. B (CCl<sub>4</sub> 1.5 ml/kg group)Fig. C (SLY 100mg/kg +CCl<sub>4</sub>)Fig. D (P.P\* 200mg/kg +CCl<sub>4</sub>)Fig. E (P.P\* 400mg/kg +CCl<sub>4</sub>)

#### IV. DISCUSSION:

Plant polyphenols have been progressively studied because of their strong antioxidant effects and their activities, theoretically avoiding numerous diseases correlated with oxidative stress, such as coronary infection, cancer, systemic inflammation, diabetes mellitus, and neurodegenerative disorders [Pandey & Rizvi., 2009]. This research indicates that the aqueous extract of *Polygonum persicaria* (Linn.) can contain substantial amounts of phenolic compounds and flavonoids and that these may be interrelated with their antioxidant function. However, as a matter of some seriousness, the recognition of the most active constituents and more thorough analyses of the biological function of the extract would have to be carried out.

The extract revealed the presence of numerous phytoconstituents such as carbohydrates, polyphenolics, alkaloids, saponins and terpenoids that are declining and non-reducing. No mortality was seen up to the dosage level of 2000 mg/kg body weight for acute toxicity.

CCl<sub>4</sub> has been commonly used for the investigation of chemical toxin-induced liver injury in animal models. Fatty liver, cirrhosis and necrosis, which have been thought to result from the development of reactive intermediates such as trichloromethyl free radicals (CCl<sub>3</sub>\*) metabolized by the diverse utility of cytochrome p450, are the most remarkable pathological features of CCl<sub>4</sub>-induced hepatotoxicity [Recknagel et al., 1989] normally the degree of hepatic damage is evaluated by the expanded degree of cytoplasmic enzymes (ALT, AST & ALP)B accordingly prompts to leakage of enormous amounts of enzymes into circulation .This was related by enormous centrilobular necrosis, swelling deterioration & cellular invasion of the liver [Gouri Shankar et al., 2008].

CCl<sub>4</sub> is an extreme hepatotoxin believed to cause hepatotoxicity in animals, close to those with serious human hepatitis [Li C et al., 2015]. CCl<sub>4</sub> is used by cytochrome P-450 and converted into trichloromethyl and trichloromethyl peroxy radicals that initiate peroxidation of polyunsaturated fat components with optional damage from different layers, extreme enzymatic administration [Zhou D et al., 2010]. Elevation of CCl<sub>4</sub>-induced serum ALT, AST and ALP production has been due to hepatic auxiliary damage because these compounds are usually confined in the cytosol and released into the bloodstream after cellular damage [Huang Q et al., 2012; Shah MD et al., 2015]. In the present investigation, the levels of all these proteins raised in CCl<sub>4</sub> administration (CCl<sub>4</sub> treated group) indicating liver damage by CCl<sub>4</sub>. The most welcoming clinical predictor of necrosis sternness is bilirubin, a product of heme degradation [Vuda M et al., 2012]. As shown in results, total bilirubin level significantly elevated in CCl<sub>4</sub> group exhibiting severe injury induced by CCl<sub>4</sub>. Pre-treatment of rats with 200 and 400 mg/kg of *Polygonum persicaria* was therefore comparable with the activity of regular silymarin (100 mg/kg) and decreased the elevated levels of ALT, AST, ALP and TB, indicating that the plant extract was effectively acting to protect rats against hepatotoxicity initiated by Carbon tetrachloride. After treatment with *Polygonum persicaria*, the restoration of serum enzyme production to normal quantities in rats demonstrates the prohibition of leakage of intracellular enzymes by preserving the integrity of the membrane of the liver cells.



Silymarin has hepatoprotective effects and is used to treat multiple diseases in the liver. [M. Elmowafy et al., 2013]. Various studies indicate that Silymarin exhibits effective antioxidant activity [R. Simeonova et al., 2013] and demonstrates defensive effects against hepatic toxicity caused by a wide variety of agents by preventing lipid peroxidation [D. Binda et al., 2001; E. Bosisio et al., 1992]. Higher total phenolic content has been recognised to add to the antioxidant actions of extract [L. Yuan et al., 2014], While antioxidant activity has also been related to the hepatoprotective effect of some extracts [L. Yuan et al., 2014; F. Gu et al., 2014]. These reports confirm with our outcomes on the capability of silymarin to utilize as a hepatoprotective activity.

The histological changes prompted by CCl<sub>4</sub> treatment as proven by centrilobular necrosis and bridging hepatic necrosis and its assurance to regularity by the treatment with the plant extract treatment is representative of the extract's hepatoprotection. The histopathological examination revealed marginal damage to a few hepatocytes in rats treated with extract in the near vicinity of the central vein and better histological scores indicated the effectiveness of the extract as an anti-hepatotoxic agent.

## V. CONCLUSION:

Taking everything into account, the subsequent effects of the current examination obviously exhibited hepatoprotective impacts of the *Polygonum persicaria* aqueous extract in CCl<sub>4</sub>-prompted hepatic harm in rats. The histopathological concentrates additionally confirm the movement of the medication. In this manner, the examination logically bolsters the treatment of this plant in different Ayurvedic arrangements and customary medication for the treatment of the liver issue and as a tonic.

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