



## Ameliorative Study of Hepatotoxicity by Medicinal Plant and Active Principle In Male Albino Rats

**Varsha Rathore**

and

**Pragya Shrivastava**

Department of Life Science,  
Rabindranath Tagore University,  
Raisen

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### Abstract

Extract of *Polygonum persicaria* Linn was evaluated for hepatoprotective and antioxidant activities in rats. The plant extract (400mg/kg/day) showed are markable hepatoprotective and antioxidant activity against Carbon tetrachloride (CCl<sub>4</sub> 1.5mg/kg i.p)-induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels in liver tissues. CCl<sub>4</sub> induced a significant rise in AST, ALT, ALP, TB. Treatment of rats with plant extract (400 mg/kg) significantly (P<0.01) altered serum marker enzymes and antioxidant levels to near normal against CCl<sub>4</sub>-treated rats. The activity of the extract at dose of 400 mg/kg was comparable to the standard drug, Silymarin (100 mg/kg, p.o.).

Keywords:-*Polygonum persicaria*, CCl<sub>4</sub>, Hepatoprotective, Silymarin.

### INTRODUCTION

Liver regulates various important metabolic functions. Hepatic damage is associated with distortion of these metabolic functions. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. This is one of the reasons for many people in the world over including those in developed countries turning complementary and alternative medicine (CAM) [Rao G.N et al., 2005]. Most of the xenobiotics enter the body through the gastrointestinal tract and after absorption enters the liver through portal vein. This organ has high concentration of toxin-metabolising enzymes which can convert xenobiotics to compounds with



low toxicity and excrete them. However, sometimes-toxic substances are converted during metabolism to active metabolites which can induce hepatic damage[Cullen2005].

Among others the mechanism of action of Carbon tetrachloride (CCl<sub>4</sub>), bromobenzene, chloroform, paracetamol, ethanol and polycyclic aromatic hydrocarbons are activated into their corresponding reactive metabolites through the action of the cytochrome P450 system mainly located in the liver in more abundant amount than in any other organ such as lung, kidney or intestine [Sheweita et al.,2001]. CCl<sub>4</sub> is one of the most commonly used hepatotoxins that induce liver injury in vivo. Inside the body, CCl<sub>4</sub> decomposes into trichloromethyl and trichloromethylperoxy highly toxic radicals (CCl<sub>3</sub>.) by the enzyme cytochrome P450. The trichloromethyl radical reacts with different significant biomolecules such as fatty acids, proteins, lipids, nucleic acids and amino acids to cause lipid peroxidation and to break DNA combination and division. therefore, causing cellular breakdown evidenced by changes in haematological and biochemical parameters [Girish et al. 2009]. Consequently, in CCl<sub>4</sub>-induced liver damages, the harmony between reactive oxygen species (ROS) fabrication and antioxidant protection system is disturbed by the oxidative stress through disrupting cellular capacities. In spite of the extensive progressions in medication, drugs utilized for liver treatment have many side effects and compound the sickness. Therefore, it is necessary to find safe alternatives from medicinal plants to replace chemical drugs, through the use of various experimental models[Muriel and Rivera-Espinoza 2008, Patwardhan et al. 2004]). In this context, more attention has been paid to the protective effects of natural antioxidants of herbal medicine and isolated bioactive constituents, which are considered as the most effective and safe treatments for hepatic toxicity[Grajales and Muriel 2015].

*Polygonum persicaria* (P.P) is a herb which belongs to the Polygonaceae family. It is locally known as Lady's thumb. This species is widely distributed mostly in Europe, North Africa, and Asian countries. These species are present in damp or low-lying areas and canal & river banks. Traditionally, the plant has acquired eminence in the treatment of wounds, menorrhagia, whooping cough, splenic diseases and fever. *Polygonum persicaria* has been accounted for to contain a few dynamic components including taxifolin, myricitrin, luteolin, proto catechuic acid, arctiin, lappal B, naringenin, etc., which were found in different phytochemical investigations [Li Y et al.,2005]. Some important pharmacological effects such as cardioprotective, antidiabetic, antioxidant and osteoblastic were observed in the plant extract [Wei Y et al., 2009; Nigam V et al.,2013; Liao SG et al.,2013; Xiang MX et al.,2011]. *Polygonum persicaria* has gotten logical consideration for its therapeutic qualities in various sicknesses; however, no examinations have been led at this point for its anti-inflammatory and pain-relieving possibilities. This plant possesses a curative potentiality for different inflammation relevant diseases, and has been used for many years in



certain communities; this traditional use makes us curious to continue our present study with this plant, and we desire to provide a scientific basis for a potential anti-inflammatory effect. We started with an in vivo investigation and continued with selected suitable animal models to determine the anti-inflammatory and analgesic activity of the aqueous extract from *Polygonum persicaria* roots. The current investigation is meant to assess the hepatoprotective and cell reinforcement action of aqueous extract of *Polygonum persicaria* against CCl<sub>4</sub>-actuated hepatotoxicity in rats.

## **MATERIALS AND METHODS**

### **Preparation of plant materials**

The plant material was collected from was collected from Bhopal near Big Pond and was identified by undersigned at centre for Biodiversity and Taxonomy, department of Botany, University of Bhopal. The dried plant materials were then pulverized into coarse powder in a grinding machine. *Polygonum persicaria* roots were aerated by cooler aeration to be partially dehydrated. They were fully dried for 7 days at room temperature. Then, the dried up roots were crush into powder, which was dissolved in distilled water and kept for another 3 days with occasional shaking and stirring. The whole combination was then filtered through Whatman filter paper, followed by filtration with a piece of clean white cotton stuff. Finally, the crude aqueous extract was preserved at 4 °C and were preliminary investigated for their biological activity.

### **Phytochemical evaluation**

The initial phytochemical screening for constituents such as steroid, alkaloid, tannin, flavonoid and glycoside in the aqueous extract of *Polygonum persicaria* was carried out following the method as described.

### **Animals**

Male wistar rats (130±10g) were used for the study. The animals were housed in well ventilated cages and given standard rat chow and water ad libitum. The animals were maintained at a controlled condition of temperature of 26-28°C with a 12 h light: 12 h dark cycle. They were acclimatized to the environment for 1 week prior to experimental use. Experiments were done according to OECD guidelines, after getting the approval of the Institute's Animal Ethics Committee (IAEC), to Pinnacle Biomedical Research Institute (PBRI) Bhopal, India(Reg.No.1824/PO/Ere/S/15/CPCSEA).

### Experimental design

Animals were divided into four groups of six rats each. Group I served as normal received only the vehicle (5% gum acacia; 1 ml/kg; p.o). Group II: Rats were induced with hepatocellular damage by receiving suspension of CCl<sub>4</sub> in olive oil. (1.5ml/kg of CCl<sub>4</sub> i.p, b.wt:1:1v/v of CCl<sub>4</sub> in olive oil) once in every day for 14 consecutive days. Group III: Rats were treated with *Polygonum persicaria* orally (through intragastric tube) at the dose of 400mg/kg body weight for consecutive 14 days. On the 14-day after 30 min post-dose of extract administration animals received CCl<sub>4</sub> at the dose of 1.5 ml/kg i.p (1:1 of CCl<sub>4</sub> in olive oil). Group IV: Standard drug as Silymarin at the dose of 100mg/kg bodyweight. Once in every day for consecutive 14 days, after 30 min post-dose of silymarin animals received CCl<sub>4</sub> at the dose of 1.5ml/kg i.p.

### Collection of Samples

On completion of the experimental period. At 24 h after last CCl<sub>4</sub> injection, blood samples were drawn from the orbital plexus, under light ether anesthesia, into non-heparinized capillary tubes. Serum was separated by centrifugation for 5 min. at 3000 rpm and stored at -20°C until analysis. The liver was isolated, washed with saline, weighed, and then 10% (w/v) homogenate of the liver was made in ice cold saline, animals were anaesthetized with thiopentane sodium (50mg/Kg). The blood was collected without EDTA as anticoagulant. Serum was separated by centrifugation and used for various biochemical analysis. A portion of liver was fixed in 10% formalin for histopathological studies.

### Statistical analysis

The values were expressed as mean  $\pm$  SD. Statistical analysis was performed by one way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. P values < 0.05 were considered as significant.

## RESULTS

The phytochemical screening of *Polygonum persicaria* showed that the presence of alkaloids, terpenoids, steroids, saponin, tannins, flavonoids, glycosides (Table-I).

Table1: Phytochemical screening of *Polygonum persicaria*

Phytochemicals	Results
Alkaloids	+

Terpenoids	+
Steroids	+
Saponin	+
Tannins	+
Flavonoids	+
Glycosides	+

+indicates presence whereas–indicates absence

The effect of *Polygonum persicaria* on serum marker enzymes is presented in Table II. The levels of serum AST, ALT, ALP, total bilirubin, were marked decreased in CCl<sub>4</sub> treated animals, indicating liver damage. Administration of *Polygonum persicaria* root extract at the dose of 400mg/kg remarkably prevented CCl<sub>4</sub>-induced hepatotoxicity in rats (Table-II).

TableII: Effect of *Polygonum persicaria* on Liver marker enzymes in experimental rats.

GROUPS	ALTIU/L	ASTIU/L	ALPIU/L	TBmg/dl
NormalControl	54.35±5.81	142.088±11.10	139.68±7.42	0.51±0.10
CCL <sub>4</sub> Toxic	209.24±10.12	287.69±60.12	345.26±74.08	2.04±0.25
P.P- 400mg/kg+CCl <sub>4</sub>	83.06±10.77	184.65±21.08	184.45±26.37	0.81±0.07
Silymarin-100 mg/kg+CCl <sub>4</sub>	56.41±14.98	150.58±15.86	151.15±12.05	0.55±0.08

Values were expressed as mean±SD for six rats in each group significantly different from Group I(p≤0.05).

## DISCUSSION

Carbon tetrachloride is one of the most normally utilized hepatotoxins in the exploratory investigation of liver ailments. The hepatotoxic effects of CCl<sub>4</sub> are to a great extent because of its dynamic metabolite, trichloromethyl radical. These activate radicals attach covalently to the macro molecules and induce peroxidative deprivation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. The ruin of lipid peroxidative of biomembranes is solitary the core reason of hepatotoxicity of CCl<sub>4</sub>, and is the evidence by an elevation in the serum marker enzymes namely AST, ALT, ALP, TB.



The conclusion of organ sickness/harm is supported by the estimation of various non-functional plasma enzymes distinguishing of that tissue or organ. The measure of compound discharged relies upon the level of cell harm, the intracellular grouping of the enzymes and the mass of influenced tissue. The concentration of the enzyme discharged reflects the harshness of the injured. ALT and AST are enzymes normally present in the liver, heart, muscles and blood cells. They are basically located within hepatocytes. So when liver cells are harmed or pass on transaminases are discharged into the circulation system, where they can be estimated they are along these lines the record of liver injury [Vasudha et al., 2006]. ALP and bilirubin levels then again are identified with the capacity of the hepatic cells. Increment in serum level of ALP is due to improved amalgamation, in occurrence of rising biliary pressure.

Administration of *Polygonum persicaria* to CCl<sub>4</sub> intoxicated rats restored the level of ALT, AST and ALP offering the maximum hepato protection with respect to different liver marker enzymes. This confirms the liver protective effect of *Polygonum persicaria*.

On the basis of above results, it can be concluded that aqueous extract of *Polygonum persicaria* is available of protection against hepatotoxicity by CCl<sub>4</sub> in animal model by minimizing biochemical parameters and tissue injury. The hepatoprotective activity of *Polygonum persicaria* perhaps because of the presence of phytoconstituents. Further investigations to portray the dynamic standards and to clarify the system are in progress.

## CONCLUSION

Finally, CCl<sub>4</sub> as one of the primary driver of hepatotoxicity prompts oxidant-antioxidant prevention agent disparity and consequently increases the action of serum biomarkers and liver tissue oxidative damages with penetration of inflammatory cells (Fig.1). CCl<sub>4</sub> toxicity cause prominent damage. Overall, it can be concluded that *Polygonum persicaria* protect liver against CCl<sub>4</sub> toxicity by antioxidant and anti-inflammatory effects.

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