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Hepatoprotective and antioxidant activity of *Persicaria maculosa* aqueous extract against carbon tetrachloride induced hepatotoxicity in Wistar rats

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The consolidated hepatoprotective effect and antioxidant activity of *Persicaria maculosa* Gray were assessed against carbon tetrachloride (CCl_4) instigated hepatic harm in Wistar albino rats. Aqueous extract of *P. maculosa* at a dosage of 400 mg kg^{-1}. Every 14 days, a portion of one's body weight was administered orally. The generously raised serum marker catalysts for example, ALT, ALP, AST, total bilirubin and the cell reinforcement proteins, for instance, glutathione reductase, glutathione peroxidase, and glutathione-S-transferase were discovered as a result of CCl_4 treatment. Following administration of plant extract, the levels of the previously mentioned enzymes were brought close to approaching regularity. At a dose of 100 mg/kg, silymarin was used as a standard reference drug in the study. The results of this study demonstrated unequivocally that *Persicaria maculosa* has a potent hepatoprotective effect in rats against CCl_4-induced hepatic damage. Histopathological changes were also seen in livers of animals that received drugs. Simultaneous organization of silymarin altogether diminished the medications-induced biochemical and histological changes toward normalcy.

Keywords: Carbon tetrachloride, Hepatoprotective, Marker enzymes, *Persicaria maculosa*, Silymarin

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Liver is an essential organ and an important role support metabolic capacity and detoxification from endogenous and exogenous problems, for example, drugs, viral diseases, xenobiotics and ceaseless liquor addiction. around 20000 deaths are because of liver issues[1,2]. Liver harm is constantly connected with cell necrosis, an expansion in interstitial fluid, peroxidative damage and decline in tissue glutathione (GSH) levels. Liver dysfunction instigate] by routine liquor intake, introduction to certain xenobiotic compounds or drug reactions. Liver disease continues to be a contentious topic[3]. Without a solid and viable operator for the avoidance as well as the treatment of liver diseases, numerous analysts are concentrating on presenting hepatoprotective combinations from natural products[4]. In this manner, therapeutic plants have as a rule been a compelling and great choice for the counteraction or treatment of liver dysfunction[5].

Today's medical technology, Various restorative arrangements are suggested in Ayurveda for the treatment of the liver conditions[6]. Taking into account serious unfortunate reactions of manufactured operators, there is a developing concentration to follow foundational research approach and to assess the logical reason for the customary natural drugs that are professed to have hepatoprotective movement. A solitary medication can't be successful for a wide range of serious liver ailments[7]. Accordingly a compelling definition must be created utilizing therapeutic plants, with legitimate pharmacological investigations and clinical preliminaries[6,8,9]. One of the most widely recognized causative elements that represents a significant clinical and administrative test is medication-induced liver injury[10].

Carbon tetrachloride (CCl_4) is the well-studied model of xenobiotic-initiated hepatotoxicity, and it is frequently used to test the hepatoprotective effects of medications and natural products. Carbon

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Abbreviations: P.P– Polygonumpersicaria, CCl_4- Carbon Tetrachloride, ALT- Alanine transaminase, ALP- Alkaline phosphatase, AST- Aspartate transaminase, OECD-Organisation for Economic Co-operation and Development, GPx- Glutathione peroxidase, GR- glutathione reductase, and GST- glutathione-S transferase, H&E- Hematoxylin Eosin, TB- total bilirubin, S- Silymarin, ROS-Reactive oxygen species.

tetrachloride (CCl_4) causes liver damage by causing trichloromethyl radicals (CCl_3• and/or CCl_3OO•) to be metabolised by cytocrome P450[11,12].

Therapeutic plants have been present subsequently the initial developments and were the principal type of treatment for infections[13]. Various Indian traditional medicines have been used to treat liver disease, and numerous studies have shown that various herbs have hepatoprotective properties[14]. The Siddha system of medicine is practised primarily in South Indian states and other South East Asian countries, and is one of the traditional Indian medicines. One of the polyherbal Siddha preparations, *Persicaria maculosa* Gray, is frequently used to diagnose and reduce liver diseases[14].

Persicaria maculosa Gray [syn. *Polygonum persicaria*] is an annual plant in the buckwheat family, Polygonaceae. Normal names incorporate woman's thumb, detected woman's thumb, Jesus plant, and redshank. This species is far reaching across in Europe, North Africa, and Asian nations. These species are found in damp or low-lying zones and trench and waterway banks. The plant has antibacterial, antifungal, and anticancer properties. *P. maculosa* aqueous root extract has antimicrobial, anticancer, and anti-inflammatory activity, according to preliminary pharmacological studies on the plant. Anticancerous activity is present in the new underlying roots of *P. maculosa*[15]. The current examination is intended to survey the antioxidant and hepatoprotective activity of *P. maculosa* aqueous extract against Carbon tetrachloride activated hepatotoxicity in rat liver.

Materials and Methods

Plant material

The new plant material *P. maculosa* was gathered from Lethpora, Pampora, Kashmir in the vicinity of River Jhelum and was identified at the University of Kashmir's Centre for Biodiversity and Taxonomy using voucher specimen Herbarium No. 2925-(KASH). The plant material was washed with water, cut into pieces, and air dried. In a grinding machine, the dried plant material was crushed into coarse powder. The plant material of 500 g was extracted in distilled water for a period of 3 days. Solvent from sample was filtered, and vanished off under decreased tension in a turning evaporator to acquire crude extract. A voucher specimen was kept in our laboratory for future reference.

Preparation of plant extracts for phytochemical analysis and hepatoprotective research

The roots of the plant were dried in the shade before being powdered with a mechanical processor to produce a coarse powder, which was then subjected to progressive extraction in a Soxhlet apparatus using distilled water. The extract was subjected to a subjective test for the identification of various phytochemical constituents in accordance with standard methodology[16-18]. The underlying phytochemical screening for constituents, for example, steroid, alkaloid, tannin, flavonoid and glycoside in the aqueous extract of *P. maculosa* was completed after the technique as depicted by Harborne[19]. In a rotating evaporator, the aqueous extract was collected. Hepatoprotective studies were conducted using the concentrated aqueous extract.

Animals used in experiments

Adult albino male rats weighing (130±10 g / 12-16 weeks old) were chosen from the departmental province and were housed in all around ventilated hardened steel confines at room temperature (24±2°C) in the sterile condition under normal light and dark schedule and were benefited from standard laboratory diet. Food and water were given ad libitum. Following approval from the Institute's Animal Ethics Committee (IAEC), the research was conducted at the Pinnacle Biomedical Research Institute (PBRI) in Bhopal, India (Reg. No.1824/PO/Ere/S/15/CPCSEA). Animals were cared for and examined in accordance with the standards proposed by the Government of India's Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Toxicity studies

The acute oral toxicity study was carried out in accordance with OECD-423 guidelines (acute toxic class method), and albino male rats (n=6) were chosen at random for the study[20]. For the time being, the animals were fasted and given only water before the extract was administered orally at 5 mg/kg body weight via gastric intubations and the animals were monitored for 14 days. If two out of three animals died, the dose was designated as toxic. When a single animal died, a similar dose was reintroduced to confirm the toxic dose. If no deaths were observed, the system was redesigned to accommodate higher dosages, such as 50, 300, and 2000 mg/kg body weight.

Experiment on hepatoprotective activity

Four groups of six rats were formed. As usual, Group I received the vehicle (5% gum acacia;

1 mL/kg; p.o). Group II: Rats were induced with hepatocellular damage by receiving suspension of CCl_4 in olive oil. (1.5 mL/kg of CCl_4 i.p, b.wt:1:1v/v of CCl_4 in olive oil) once in every day for 14 consecutive days. Group III: Rats were treated with *P. maculosa* orally (through intragastric tube) for 14 consecutive days at a dose of 400 mg/kg body weight. On the 14th day, 30 min after the extract administration, the animals were given 1.5 mL/kg i.p., of CCl_4 (1:1 of CCl_4 in olive oil). Silymarin at a dose of 100 mg/kg body weight is used in Group IV. After 30 min of silymarin administration, animals were given 1.5 mL/kg i.p., of CCl4 once a day for 14 days. The rats were anaesthetized with ether one day after their last treatment, and by using the retro-orbital puncture technique, blood samples were collected in tubes for biochemical analysis. To separate the serum, blood tests were centrifuged for 10 min at 3000 rpm. The animals were euthanized under ether anaesthesia after blood was collected, and liver tissue was collected for histopathological studies.

Analyses of biochemical parameters

The animals were relinquished toward the finish of the test time of 14 days by the cervical dislocation method. Blood was gathered, serum isolated by centrifugation at 3000 rpm for 10 min. Aspartate transaminase (AST)[21], Alanine transaminase (ALT)[21], Alkaline phosphatase (ALP)[22] & Total bilirubin[23]. Standard methods were used to determine levels in serum proteins.

The extent of tissue toxicity, on the other hand, is communicated as far as GPx[24]. (Glutathione peroxidase), glutathione reductase (GR)[25], and glutathione-S transferase (GST)[26]. On the *'in vivo'* subjects, they were subjected to the standard techniques for assessing oxidative stress**.**

Histopathological analysis

Fresh tissue pieces of liver were fixed in 10% formalin for proper fixation before being examined at the light microscopic level. The specimens were washed and dehydrated in an ascending series of ethanol (70–100%) after two days of fixation. They were cleaned with xylene and embedded in paraffin wax before being sectioned with a rotary microtome at a thickness of 5 µm. After being rehydrated in distilled water, the sections were stained with Hematoxylin and Eosin (H&E) and examined under a light microscope.

Statistical evaluation

The information was presented in the form of mean standard deviation (SD). For statistical analysis, one way analysis of variance (ANOVA) was used, followed by the Bonferroni t-test. p values of ≤ 0.001 were deemed significant.

Results

The presence of alkaloids, phenols, steroids, saponins, tannins, flavonoids, and glycosides was found in an aqueous extract of the root portion of *P. maculosa* subjected to a phytochemical study. The aqueous extract exhibited no toxicity or mortality up to a dose of 2000 mg/kg.

When CCl4 intoxicated group II was compared to normal group I, there was a significant ($p \leq 0.001$) increase in serum ALT, AST, ALP, and total bilirubin levels. Aqueous extract of *P. maculosa* (Group III) at dosage of 400 mg/kg essentially diminished raised serum indication chemicals & switched to practically ordinary levels. Standard silymarin (Group IV) showed the most significant decreases in concentration, followed by (Group III) Table 1.

The impact of aqueous extract of *P. maculosa* on glutathione peroxidase, Glutathine-S-transferase & glutathione reductase action is appeared in Table 2. Glutathione peroxidase, Glutathine-S-transferase & glutathione reductase, when CCl_4 intoxicated rats were compared to the animals in the normal control group, their activity was significantly ($p \leq 0.001$) decreased. Rats given 400 mg/kg of *P. maculosa* aqueous extract fundamentally the levels of modified Glutathine-S-transferase, glutathione peroxidase, and

Table 1 — The effect of *P. maculosa* aqueous extract on liver AST, ALT, ALP, and TB levels in normal, liver-injured, and drug-treated rats is shown in Table 1.

Groups	Treatment	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	BIL (mg/dL)
I	Normal-control	49.05±7.821	135.69±11.761	117.18±7.494	0.41±0.067
II	CCl_4*- Negative	76.90±4.449	168.8±12.95	222.11±27.16	2.00±0.062
III	P.M* 400 mg/kg+CCl_4	58.34±5.589	150.3±9.467	145.16±7.304	0.65±0.075
IV	SLY* 100 mg/kg +CCl_4	47.73±8.294	132.16±19.97	113.22±5.905	0.46±0.056

The results were expressed as Mean S.D. (n= 6) with $p \leq 0.001$ versus the control group of rats and $p \leq 0.001$ versus the CCl_4 induced group of rats. P. M*- *Persicaria maculosa*, SLY*- Silymarin. CCL_4*- carbon tetrachloride.

Table 2 — shows the effect of therapeutic agents on antioxidant enzyme activity.

Paramters	Control	CCl_4	CCl_4+PM	CCl_4+S
GST (μ mole/min/protein)	8.09±0.56	3.71±0.29$^{\$}$	6.10±0.44$^{@}$	7.47±0.56*
GPx (μ mole/min/protein)	6.11±0.45	3.24±0.18$^{\$}$	4.61±0.28$^{@}$	5.17±0.36*
GR (μ mole/min/protein)	4.37±0.26	2.50±0.19$^{\$}$	3.60±0.23$^{@}$	4.12±0.23*

Data are mean S.D., N = 6; @ =Significant at p≤0.001 for ANOVA; $^{\$}CCl_4$ at p≤0.001; $^{@}CCl_4$+ Therapy vs$^{*}CCl_4$ at p≤0.001. S –Silymarin

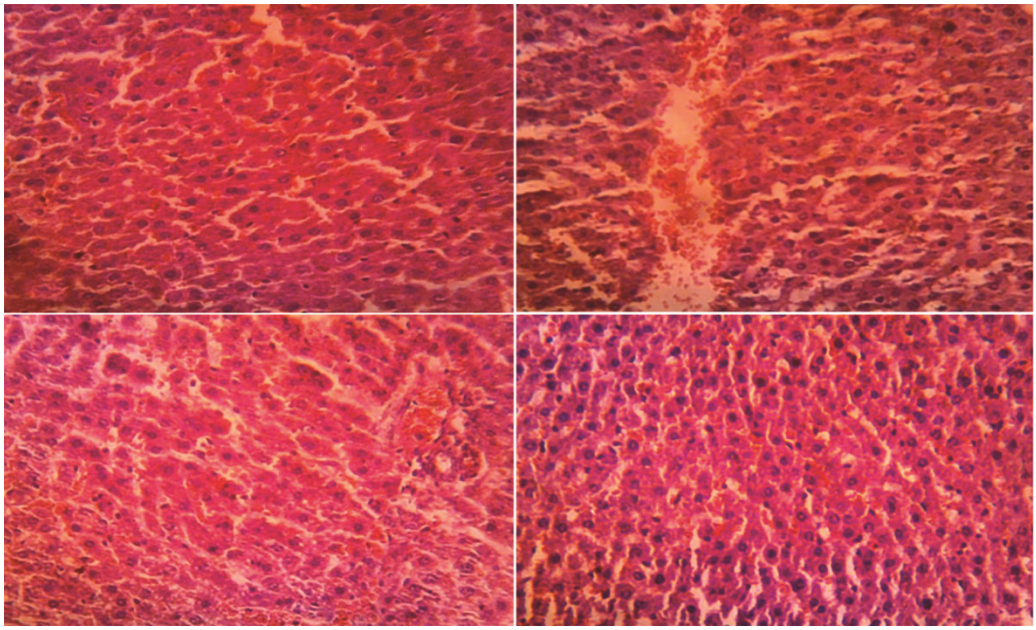

Fig. 1 — (a) (Normal group-I), (b) ($CCl_4$1.5 mL/kg i. pgroup-II), (c) (P.P 400 mg/kg+CCl_4 group-III), (d) (SLY100 mg/kg +CCl_4 group-IV)

glutathione reductase were restored to normal. The results are nearly identical to those obtained in the silymarin (standard drug)-treated group.

Studies on histopathology

Cell injury caused by CCl_4 was discovered in the livers through histopathological examinations. Ordinary control hepatocytes had a typical design showing normal hepatic parenchyma with hepatic lobules and occasional cytoplasmic vacuolation in photomicrographs of hematoxylin and eosin tainted liver tissues (Fig. 1a). 24 h after CCl_4 treatment, rats showed severe fatty degeneration, vacuolation, and hepatocyte necrosis (Fig. 1b). The harshness of CCl_4 inflicted liver injury when an aqueous extract of *P. maculosa* was pretreated at 400 mg/kg body weight. Typical hepatic parenchyma with hepatic lobules and cytoplasmic vacuolation (Fig. 1c). The effects of silymarin (100 mg/kg body weight) on the histopathology of the livers of CCl4-treated rats have been demonstrated (Fig. 1d). The liver has a better structural appearance with no hepatocyte necrosis. The outcomes obviously demonstrate the insurance gave by the powerful antioxidant aqueous extract of *P. maculosa*.

Discussion

The presence of flavonoids and phenolic compounds, both of which have been linked to antioxidant and hepatoprotective properties, was discovered during preliminary phytochemical analysis of the extract. Saponins in *P. maculosa* aqueous extract are thought to play a key role as an antioxidant that protects the liver from oxidative damage. Furthermore, the flavonoids and saponins in *P. maculosa* aqueous extract may be able to counteract reactive oxygen species by reacting with them and oxidising them to less reactive radicals. Our findings back up reports of this aromatic plant being used to treat liver diseases and jaundice in traditional medicine. More research is being conducted to regulate which phyto-constituents are responsible for the hepatoprotective effect.

It is well known that CCl_4 causes hepatotoxicity by initiating the metabolic process; as a result, it causes toxicity only in liver cells with a semi-normal

metabolic capacity. The endoplasmic reticulum framework's cytochrome P-450 enzyme converts CCl_4 to trichloromethyl free radical [CCl_3]. Trichloromethyl free radical then reacted with cell lipids and proteins in the presence of oxygen to form trichloromethyl peroxyl radical, which can attack endoplasmic reticulum layer lipids faster than trichloromethyl free radical. As a result, the trichloromethyl peroxyl free radicals cause lipid peroxidation, which, in turn, causes Ca^{2+} homeostasis disruption and, ultimately, cell death[22,23]. There are changes in the structures of the endoplasmic reticulum and another layer, as well as a loss of chemical metabolic catalyst initiation, a decrease in protein blend, liver damage due to a loss of glucose - 6-phosphate activation[27,28].

Hepatotoxic compounds, such as CCl_4, have been shown to significantly increase serum enzymatic activities. Treatment with *P. maculosa* plant extract reduced CCl_4-induced increases in the activities of aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP), indicating that *P. maculosa* plant extract protects against CCl_4-induced liver injury.

Alkaline phosphatase is a model of these proteins that reflects the pathological change in biliary flow[29]. The CCl_4-induced increase in this enzymatic movement in serum corresponds to an increase in serum bilirubin content. The fact that the aqueous extract of *P. maculosa* initiated concealment of the increased ALP action while simultaneously exhausting raised bilirubins suggests that the extract has the potential to stabilise bile duct dysfunction in the rat liver during CCl_4-induced hepatic damage. Along these lines organization of aqueous extract of root portion of *P. maculosa* is against harmful impact of CCl_4.

Bilirubin is a yellow pigment produced when heme is catabolized. Hepatocytes render bilirubin water-solventand by conjugating it with glucuronic acid before discharging it via active transport into bile, it is effectively excretable. The production of more bilirubin than the liver can process, liver damage that impairs the liver's ability to excrete a normal amount of bilirubin, or an obstruction of the liver's excretory pipes can all lead to hyperbilirubinemia[30]. Serum bilirubin is regarded as a reliable indicator of liver capacity because it reflects the liver's ability to absorb and process bilirubin into bile. A few diseases may be manifested by elevated levels. CCl_4 toxicity could be predicted by significant levels of complete bilirubin in CCl_4-treated rats. As a result, hyperbilirubinemia may have developed. The fact that total bilirubin levels in the plant extract-treated serum were significantly lower suggested that the plant extract may have hepatoprotective properties against CCl_4 intoxication.

The body has a suitable framework to keep away from and kill the free radical provoked injury. This is developed by a lot of endogenous malignant growth avoidanceoperator catalysts, for example, glutathione peroxidase, glutathione reductase and glutathione S transferase. Exactly when the harmony between ROS generation and cell reinforcement defend is lost, oxidative pressure results, which through a movement of events deregulates the cell limits advancing diverse dreadful conditions[31]. Any compound, typical or fabricated, with cancer prevention agent properties may contribute towards the fragmentary or complete speeding up of this sort of injury. In the current examinations, CCl_4 intoxicated rats had lower levels of GPx, GR, and GST in their livers, whereas treatment with *P. maculosa* aqueous extract 200 mg/kg was able to reverse these effects.

In addition, the CCl_4-treated rat's liver sample was histologically examined, revealing chronic necrosis. The administration of aqueous extract of *P. maculosa* 200 mg/kg and silymarin 100 mg/kg significantly reduced serious liver injury caused by CCl_4, as evidenced by the nearness of normal cell limits, less greasy changes, absence of necrosis and swelling degeneration, and extensive lymphocyte invasion.

Conclusion

The consequences of this investigation exhibit that the aqueous extract of *P. maculosa* has an intense hepatoprotective activity against CCl_4 instigated hepatic damage in rats. Cell membrane changes, hepatic cell recovery, and the production of antioxidant enzymes like glutathione peroxidase, glutathione reductase, and glutathione S transferase could all play a role in its ability to manage the hepatoprotective response to CCl4-induced liver damage. The hepatoprotective and antioxidant potential of plant extract could be attributed to various phytochemical standards found in *P. maculosa*, such as flavonoids, alkaloids, phenolics, and tannins. As a result of this study's findings, *P. maculosa* provides significant protection against CCl_4-induced hepatotoxicity.

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Conflict of Interest

During the research, the authors declare that they had no commercial or financial relationships that could be considered a potential conflict of interest.

Authors' Contributions

MS, DK conceptualized; MS, DK compiled data; MS, DK formalized; MS, DK methodology; MS, DK, AT MS manages the project. Writing–first draught: MS; MS, DK, and AT wrote–reviewed and edited.

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