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EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF POLYGONUM PERSICARIA (LINN.) AGAINST CCL_4 INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The current studies were directed to test the antioxidant and hepatoprotective ability of the root section of Polygonum persicaria (linn). The hepatoprotective function of the aqueous extract of Polygonum persicaria (PP) was examined by utilizing carbon tetrachloride (CCl_4) inebriation in rats as test models. For 14 days, rats were randomly assigned to one of five categories: normal control, toxic control (1.5ml/kg CCl_4), standard control (Silymarin 100mg/kg), and experimental groups of Polygonum persicaria (200 & 400 mg/kg b.w p.o). The existence of secondary metabolites was discovered by a phytochemical screening of *Polygonum persicaria*. *Polygonum persicaria* aqueous extract was tested for antioxidant activity using a DPPH radical scavenging assay. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as other biochemical parameters. Alkaline phosphatase (ALP), bilirubin (BIL), and total protein (TP) in the blood serum and reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione s-transferase (GST) concentrations were used to determine liver damage and the plant extract impact. The liver's histopathological modifications in different groups were also investigated. This investigation demonstrates that the hepatoprotective effect and antioxidant activity of the root part of Polygonum persicaria therefore scientifically supports the therapy of liver diseases against CCl_4.

KEYWORDS: Antioxidant, Carbon tetrachloride, Hepatoprotective, Histopathology, *Polygonum persicaria*, Silymarin.

1. INTRODUCTION

The liver is involved in the management of many biochemical functions, and most hepatic diseases are caused by excessive alcohol use, radiation toxicity, autoimmune conditions, and viral infections. Carbon tetrachloride (CCl_4) is a toxic chemical that causes lipid peroxidation (LPO) and oxidative stress in liver cells.[1-5] Excessive reactive oxygen species (ROS) in the liver can cause severe liver injury.[6] Oxidative stress induces cell injury by creating reactive oxygen species (ROS). Antioxidant agents can be an effective tool to counteract oxidative stress that causes liver damage and diseases, based on the essential pathophysiological role of antioxidant/detoxifying malfunction in the production of liver disease.[7] Cells have developed adaptive, dynamic mechanisms to maintain cellular redox homeostasis and minimise oxidative damage in response to oxidative stress caused by ROS by a sequence of antioxidant molecules and detoxifying enzymes.[8] LPO is involved in the pathogenesis of CCl4-induced hepatic damage and is activated by the P450 enzyme system, which produces highly reactive trichloromethyl radicals.[9] Glutathione (GSH) and protein thiols can be reacted with these trichloromethyl radicals. CCl_4 also affects antioxidant enzymes including glutathione reductase (GR), thiobarbituric acid reactive substances (TBARS), glutathione peroxidase (GPx), catalase (CAT), glutathione-S-transferase (GST) and superoxide dismutase (SOD).[10] In the liver microsome during ethanol metabolism cirrhosis and hepatitis can be caused by enriched LPO produced.[11] Viruses cause approximately 90% of acute hepatitis, according to a WHO survey,[12] and it is also the world's seventh leading cause of death.

Metabolomics has been generally used to consider sub-atomic pathways in various fields, including drug digestion, medications, toxicology, etc.[13,14] Likewise, metabolomics may give helpful markers to DILI expectation.[15] Pannala et al. utilized metabolic specialists to recognize acetaminophen-incited poisonousness markers in rats and found that nucleotide, lipid, and amino corrosive digestion were the main pathways.[16] Hydrazine changed the digestion pathways of amino acids, glutathione metabolites, nutrients, and unsaturated fats, as indicated by serum and urine metabolomics investigation of hydrazine-treated and control rats.[17]

In recent years, due to advances in modern pharmacology and molecular biology. Natural items and compounds' potential for the prevention and treatment of human diseases has received a lot of attention The genus *Polygonum* (Polygonaceae) contains around 300 species that are generally dispersed around the earth, for the most part in north temperate regions.

Among 113 *Polygonum* species in China[18], *Polygonum cuspidatum* Sieb et Zucc., *P. aviculare* L., *P. bistorta* L., and *P. flaccidum* Meissn have been utilized as popular Chinese drugs, as described in the Chinese Pharmacopoeia. Additionally, *P. orientale* L., *P. tinctorium*, *P. persicaria* L., and *P. runcinatum* have been appeared to have great impacts as conventional Chinese medications.

Polygonum plants have been researched for their chemical components and pharmacological practises since the last century. Phytochemical experiments have shown the existence of flavonoids, quinones, phenylpropanoids, and terpenoids in the genus Polygonum in recent years, due to this rapid advancement of chromatographic separation technology and experimental methods. Furthermore, anticancer, antitumor, antioxidative, reducing, pain-relieving, antimicrobial, and insecticidal activities have been found in rudimentary extracts and pure compounds from this species. Polygonum persicaria (linn.) has been proposed in Ayurveda and is called as Lady's thumb. Anti-inflammatory[19] and antidepressant[20] effects have been studied. Polygonum persicaria contains several compounds of biological interest, including the broad spectrum of flavonoids[21] and polyphenols.[22] *Polygonum persicaria* is scattered worldwide and found in Indo-China, and Malesia; Europe regions.[23] The plant frequently develops in wet regions at watersides, side of the road, and in swamps.[24] As a result, the current research was designed to determine the antioxidant and hepatoprotective properties of Polygonum persicaria root aqueous extract in vitro and in vivo against CCl_4-induced toxicity.

2 MATERIALS AND METHODS

2.1 Plant material and preparation of the extract

The root parts of *Polygonum persicaria* were collected from Lethpora, Pampora, Kashmir in the vicinity of River Jhelum in July. With voucher specimen Herbarium No. 2925 (KASH), was identified at the Centre for Biodiversity and Taxonomy, Department of Botany, University of Kashmir. The dried roots were macerated in distilled water for 7 days (1:10 drug:solvent ratio). Subsequently, the material was filtered through cotton and concentrated in a rotatory evaporator at reduced pressure, at a maximum temperature of 40 ^{0}C, until a soft extract (90% of dry residue) of Polygonum persicaria roots (aqueous extract) was obtained.

2.2 Chemicals

All the chemicals and solvents were used of the highest purity, commercially available, and analytical grade. SICCO Research Laboratory, Mumbai, India, provided carbon tetrachloride.

2.3 Phytochemical screening

The presence of phytochemicals in the Polygonum persicaria extract was qualitatively examined using standard methods.[25]

2.4 In-vitro Free radical scavenging activity

2.4.1 DPPH radical scavenging activity

The antioxidant activity of Polygonum persicaria plant extract was determined by its ability to scavenge free radicals. 1-diphenyl-2-picryl-hydrazyl (DPPH) is a stable free radical.[26] In methanol, a 0.1 mM DPPH solution was prepared. 1 ml of this solution was added to 2 ml of reference drug solution at varying concentrations (20–100 µg/ml). The mixture was shook vigorously before being set aside at room temperature for 30 minutes. At 517 nm, the absorbance was measured. Ascorbic acid was used as a control. The following formula was used to calculate the percentage of scavenging activity:

Percentage of inhibition (%) = $A_{control} - A_{sample} / A_{control} \times 100$ (1)

Where, $A_{control}$ − absorbance of DPPH, A_{sample} − absorbance of DPPH with test sample.

2.5 In-vivo CCl_4 induced hepatotoxicity in rats

The preventive effect of Polygonum persicaria therapy against CCl_4-induced acute hepatotoxicity in rats was tested in a 14-day experiment.

2.6 Experimental animals

Male Wistar albino rats weighing 130±10 g were used in this study. The animals were acclimatised to laboratory conditions at room temperature and maintained under standard conditions with a 12 hour light/dark cycle prior to experiments. Rats were fed a daily pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water ad libitum in polyacrylic cages. The experimental procedure was approved by the Institutional Animal Ethics Committee (IAEC) and carried out at the Pinnacle Biomedical Research Institute (PBRI) Bhopal, India (Reg.No.1824/PO/Ere/S/15/CPCSEA) in accordance with the recommendations of the Committee for Control and Supervision of Experiments on Animals (CPCSEA) of the Government of India on animal experimentation.

2.7 Acute Toxicity Studies

The OECD-423 protocols were used to do the acute oral toxicity study (acute toxic class method). For the study, male Albino rats (n=3) were chosen at random.[27] The animals were fasted for an overnight time and given only water before receiving the extracts orally at a

dosage of 5 mg/kg body weight through gastric intubations and being observed for 14 days. The dosage given was listed as toxic if two out of every three animals died. When one animal died, the remaining animals were given the same dosage to verify the toxic dose. The procedure was replicated with higher doses of 50, 300, and 2000 mg/kg body weight if there was no sign of mortality.

2.8 Experimental protocol and procedure

Rats were divided into five main groups, each with six animals. Group I was given distilled water (1 ml/kg body weight, p.o). Group II were given CCl_4 (1.5 ml/kg body weight, i.p.) mixture of CCl_4 with olive oil [1:1 (v/v)]. Group III was given the standard medication silymarin (100 mg/kg body weight, p.o) For 14 days[28] as well as a CCl_4–olive oil mixture (1:1, 1.5 ml/kg body weight, i.p). *Polygonum persicaria* doses of 200 and 400 mg/kg (p.o) body weight were given to groups IV–V (test group animals) for 14 days. They were also given a dose of CCl_4–olive oil mixture (1:1, 1.5 ml/kg, i.p) 30 minutes after receiving Polygonum persicaria. On day 15, the animals were anaesthetized with ethyl ether, and blood was extracted by retro-orbital puncture, which was then allowed to clot until serum was separated for enzyme activity testing. The rats' livers were dissected and rinsed with ice-cold isotonic saline after they were disabled by cervical dislocation (0.9 percent sodium chloride).

2.9 Biochemical Analysis

The collected blood samples were centrifuged at 3000 rpm for 15 min at 4 °C. The levels of Alanine aminotransferase (ALT)[29], aspartate aminotransferase (AST)[29], alkaline phosphatase (ALP)[30], bilirubin (BIL)[31], and total protein (TP)[32] were quantitatively analysed using a Glamour 4000 biochemical analyzer (Glamour Co., Ltd., United States).

2.10 Assessment of GSH, GPx, GR, GST, G6PDH activity

Glutathine-S-transferase, glutathione reductase,[33], glutathione peroxidase[34] and glucose-6-phosphatase dehydrogenase.[35] The degree of hepatic GSH was evaluated using Ellman's (1959)[36] method with minor modifications. In a nutshell, liver tissue was homogenised in a mixture of 10% TCA and 10 mM EDTA (in a 1:1 ratio, 10% w/v). The samples were centrifuged at 3000 rpm for 10 minutes at 4 0C. 100 mL supernatant and 50 mL Ellman's reagent were combined in a container (DTNB solution). After raising the volume to 1 ml with Tris–HCl pH 7.4, the yellow colour produced was measured at 412 nm.

2.11 Histopathology

After bleeding, fresh liver tissues were fixed in Neutral-buffered formalin 10%, embedded in paraffin, and sliced into 5 µm pieces. The tissue sections were then stained with hematoxylin and eosin (H & E) for histopathological examination.[37] The sections were examined under a microscope for histopathological alterations, necrosis, steatosis, and fatty changes in hepatic cells.

2.12 Statistical analysis

All values were expressed as mean ± SD (n = 6 in each group). One-way ANOVA was applied to test for the significance of biochemical data of the different groups. Significance is set at $p \leq 0.001$.

3. RESULTS

3.1 Phytochemical composition of the extract

Table 1 shows preliminary phytochemical screening of Polygonum persicaria (P.P) aqueous extract.

Aqueous Extract	Phytochemical tests						
	Flavonoids	Tannins	Proteins	Carbohydrates	Terpenoids	Alkaloids	Saponins
	+	+	+	+	+	+	+

(+) Present, (−) Absent.

3.2 Antioxidant potential of Polygonum persicaria root extract

P.P was tested for its ability to scavenge DPPH free radicals, and the findings are shown in table 2. As a result, DPPH is a stable free radical with a characteristic absorption at 517 nm. Antioxidants scavenge the free radical by hydrogen donation, causing the colour of the DPPH assay solution to lighten and the absorbance to decrease. P.P was found to be a potent DPPH free radical scavenger, and this activity was 29.34%, 41.41%, 51.57%, 68.85%, and 75.29%, respectively, for 20, 40, 60, 80 and 100 µg /ml of P.P. The IC_{50} value was calculated to be 54.48 µg /ml, and it was comparable with that of ascorbic acid (IC_{50} 37.62 µg /ml).

3.3 Table 2: DPPH scavenging activities of extract of *Polygonum persicaria* and ascorbic acid as control.

concentrations µg ml^{-1}	% inhibition	
	Polygonum persicaria	**Ascorbic acid**
20 µg ml^{-1}	29.34	40.58
40 µg ml^{-1}	41.41	50.58
60 µg ml^{-1}	51.57	62.98
80 µg ml^{-1}	68.85	71.74
100 µg ml^{-1}	75.29	79.59
	IC_{50} = 54.48 µg ml^{-1}	IC_{50} = 37.62 µg ml^{-1}

Values were expressed as mean ± SD for triplicates

3.4 Acute toxicity

The administration of Polygonum persicaria aqueous extract to rats at various oral doses of 5, 50, 300, and 2000 mg/kg/body weight did not cause drug-related toxicity or mortality in the animals, and it was safe up to the dosage of 2000 mg/kg/body weight.

3.5 Table 3: Acute toxicity of aqueous extract of *Polygonum persicaria*.

Oral Doses (mg kg^{-1} b.wt)	Mortality
	Polygonum persicaria
5 mg kg^{-1}	Nil
50 mg kg^{-1}	Nil
300 mg kg^{-1}	Nil
2000 mg kg^{-1}	Nil

3.6 Table 4. Effect of *Polygonum persicaria* aqueous extract on CCl_4-induced hepatotoxicity-related parameters in rats.

Treatment group	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	BIL(mg/dl)	TP (g/dl)
Control	135.69±11.761	49.05 ± 7.821	117.18 ± 7.494	0.41 ± 0.067	7.26±0.092
CCl_4 1.5 ml/kg	168.8 ± 12.95	76.90 ± 4.449	222.11 ± 27.16	2.00 ± 0.062	4.68±0.160
S* (100mg) +CCl_4	132.16 ± 19.97	47.73 ± 8.294	113.22 ± 5.905	0.46 ± 0.056	7.29±0.106
P.P* (200 mg) +CCl_4	157.35 ± 9.242	64.97 ± 6.827	163.94 ± 9.248	1.085 ± 0.042	5.27±0.243
P.P* (400 mg) +CCl_4	150.3 ± 9.467	58.34 ± 5.589	145.16 ± 7.304	0.65 ± 0.075	5.86±0.262

All values were expressed as mean ± SD (n=6). Statistically significant of P≤0.001 compared to Normal control group (I), and P≤0.001 compared to CCl_4 treated group (II). S*-Silymarin; P.P* - *Polygonum persicaria*, AST-aspartate aminotransferase, ALT- alanine aminotransferase, ALP- alkaline phosphatase & BIL-bilirubin were significantly (p≤0.001) increased; however, a significant (p≤0.001) decrease in the levels of total protein (TP).

Table 4 shows the effects of P.P on biochemical markers widely used to evaluate acute hepatic damage in CCl_4-intoxicated rats. In the hepatotoxic model group-II, serum activities of ALT, AST, ALP, and BIL enzymes were significantly increased ($p \leq 0.001$) relative to the normal control group-I) after a single injection of CCl_4 (1.5ml/kg i.p). In comparison to group-I, the TP level in group-II decreases. These biochemical parameters were all elevated, indicating that hepatic cells had been damaged. When animals were pre-treated with various doses of P.P (groups IV–V; 200 & 400 mg/kg b.w.) for 14 days, the activities of serum AST, ALT, ALP, and BIL were greatly decreased, while the amount of TP increased ($p \leq 0.001$). The effect was shown to be dose-dependent, but given the large number of animals in each sample, the large standard deviation (SD) in all pre-treatment groups still demonstrated the persistence of interindividual differences in drug reaction between live animals.

3.7 Table 5. Effect of *Polygonum persicaria* on hepatic antioxidant enzymes against CCl_4.

Groups	GSH	GPx	GST	GR	G6PDH
Control	8.5±0.50	6.10±0.43	8.08±0.54	4.36±0.24	10.2±0.51
CCl_4 (1.5 ml/kg)	4.39±0.44	3.21±0.15	3.70±0.27	2.49±0.17	5.41±0.36
S (100mg/kg) + CCl_4 (1.5 ml/kg)	8.89± 0.29	5.15±0.33	7.45±0.52	4.10±0.20	9.80±0.55
PP (200mg/kg) + CCl_4 (1.5 ml/kg)	6.80± 0.34	4.58±0.24	7.23±0.43	3.59±0.20	8.49±0.73
PP (400mg/kg) + CCl_4 (1.5 ml/kg)	8.20± 0.45	4.83±0.37	7.25±0.44	3.90±0.33	9.38±0.50

Data are mean ± S.D., N = 6; @ =Significant at $P \leq 0.001$ for ANOVA; CCl_4 vs C at $P \leq 0.001$; CCl_4+ Therapy vs CCl_4 at $P \leq 0.001$. Abbreviations: CCl_4= Carbon tetrachloride; S= Silymarin; PP= Polygonum Persicaria; Values are expressed as GSH, glutathione (μ mole/min/mg protein); GPx, glutathione peroxidase (μ mole/min/mg protein); GR, glutathione reductase (μ mole/min/mg protein); GST, glutathione-S-transferase (μ mole/min/mg protein); G6PDH**,** glucose-6-phosphatase dehydrogenase (μ mole/min/mg protein).

We studied the natural antioxidant cell defences of Polygonum persicaria, including the enzymes GSH, GPx, GR, GST, and G6PDH, in the livers of animals to learn more about the mechanism of defence against CCl_4 hepatotoxicity. In comparison to normal animals (Group I), the activities of their tissue antioxidant enzymes decreased dramatically after CCl_4 (Group II) induced hepatic harm. Oral administration of PP at a dosage of 400 mg/kg (Group V) resulted in substantial increases in GSH, GPx, GR, GST, and G6PDH, when contrasted to

CCl_4-treated rats. In comparison to CCl_4-treated rats, treatment with PP at 200 mg/kg (Group IV) resulted in only a slight rise in the amount of antioxidant enzymes in liver homogenate (Table 1). When contrast to the control group, there was a substantial inhibition in GSH, GPx, GR, GST, and G6PDH activities after 14 days of CCl_4 intoxication.

3.8 Histopathological studies

The architecture of group I (Figure 1a) animals were normal histopathologically. The natural architecture of the liver was totally destroyed in CCl_4-treated rats (Group II), with the introduction of centrilobular necrosis. In addition to the swollen nuclei, scattered masses of necrotic tissues were included in most of the fields (Figure 1b). The architecture of silymarin-treated rats (Group III) was nearly normal, with uniform sinusoids (Figure 1c). Pre-treatment with PP (Groups IV and V) significantly reduced the CCl_4-induced liver damage, as well as the normal histological improvements in the liver parts (Figure 1d and 1e).

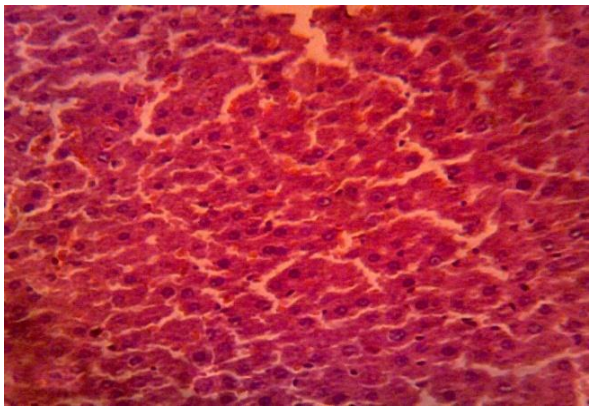

Figure 1a (Normal group-I)

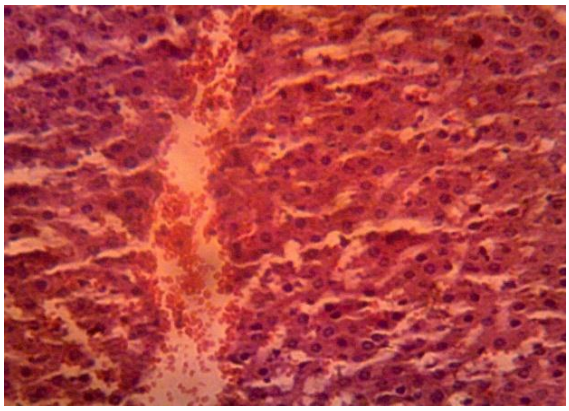

Figure 1b (CCl_4 1.5 ml/kg group-II)

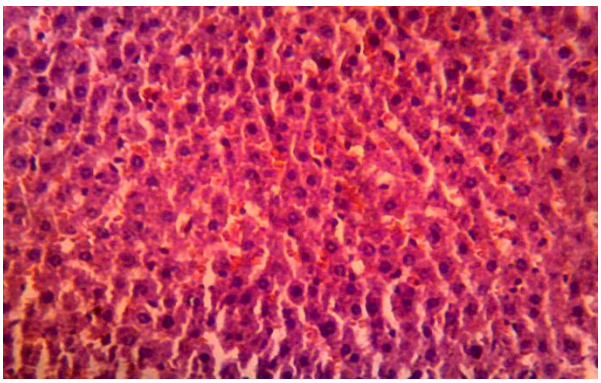

Figure 3c [SLY.100mg/kg+CCl_4 group-III)

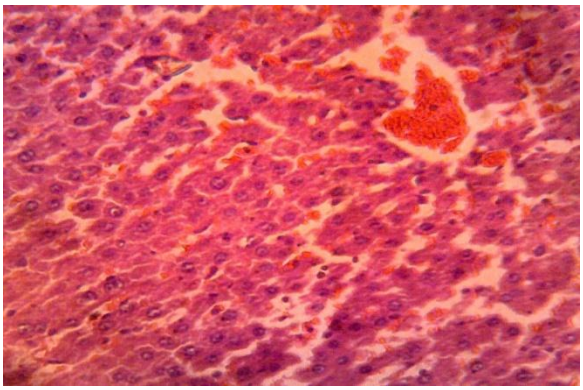

Figure 1d (*P.P.*200mg/kg+CCl_4 group-IV)

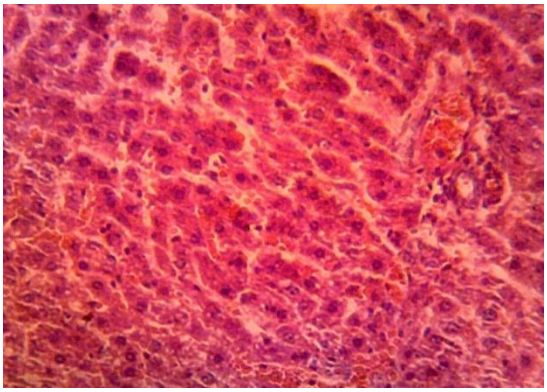

Figure 1e (*P.P.*400mg/kg+CCl_4 group-V)

4. DISCUSSION

A large number of people especially the rural people depend on natural resources for their survival. The study of cultural uses of medicinal plant species is significant to contemporary medicine. The liver is responsible for the detoxification of chemicals and drugs. This makes it the target organ for all toxic chemicals because of its vital and unique functions. Any injury or impairments of its function may lead to several implications on health. Metabolic processes are primarily controlled by the liver and its disruption contributes to liver injury.[38] Due to the lack of a reliable liver protective drug in conventional medicine, Ayurveda recommends a number of herbal formulations for the treatment of liver diseases.[39] Natural ingredients are considered safe and beneficial alternative medicines, according to some reports. Natural drugs have made significant contributions to drug development because they have many benefits over chemical compound-based drugs, including less adverse effects, lower long-term toxicity, and high bioavailability and biological activity.[40]

The experimental model used in this analysis was CCl_4-mediated hepatotoxicity. Since the modifications caused by CCl_4 liver damage are similar to those caused by acute viral hepatitis.[41] The capacity of a hepatoprotective medication to reduce the adverse effects of a hepatotoxin or to restore natural hepatic physiological processes that have been disrupted is an indicator of its protective effects.[42] The hepatotoxicity caused by CCl_4 results in lipid peroxides, resulting in hepatic damage, due to its free radical metabolite CCl_3•, which alkylates cellular proteins and other macromolecules while simultaneously attacking polyunsaturated fatty acids in the presence of oxygen.[43] The capacity of a hepatoprotective medication to reduce the adverse effects of a hepatotoxin or to restore natural hepatic physiological processes that have been disrupted is an indicator of its protective effects.[44]

CCl_4 causes laboratory damage that appears like viral hepatitis histologically. Hepatotoxicity begins with a transition in the endoplasmic reticulum, which causes metabolic enzymes to be released from intracellular structures. The peroxidation of lipids, covalent binding of macromolecules, disruption of metabolic pathways in mitochondria, decrease in phospholipid levels, rise in triglyceride levels, and inhibition of calcium pumps in microsomes result in liver necrosis.[45] The acute toxicity analysis (LD_{50}) of the aqueous extract of Polygonum persicaria was greater than 2000mg/kg, meaning that the extract was relatively safe, according to the findings of this study.[46]

The determination of the serum level of cytoplasmic enzymes such as ALT, AST, and ALP, which leak from damaged liver cells into the blood and signify centrilobular necrosis, ballooning degeneration, and cellular infiltration, is one method for estimating the extent of hepatic harm.[47] CCl_4 intoxication significantly raised the serum levels of ALT, AST, and ALP in the animals in this study, a marker of cellular leakage and deficiency of cell membrane activities in the liver.[48] The presence of CCl_4 in the serum reduces the concentration of TP, which is another sign of liver toxicity. Endoplasmic reticulum polyribosomes are disrupted and dissociated by CCl_4, resulting in a decrease in protein synthesis.[49] The *Polygonum persicaria* extract (200 and 400 mg/kg/d) & silymarin (100 mg/kg/d) reversed the elevated levels of ALT, AST and ALP induced by CCl_4 toxicity. Protein concentration measurements were primarily used to determine a protein's purity level. CCl_4 induces TP depletion, which indicates tissue injury, as seen in our research. The *Polygonum persicaria* extract and silymarin restored the antioxidant enzyme as well as the TP and reduced the CCl_4-induced oxidative injury, thus proving its antioxidant potential. In this respect, we discovered that the extract raised the elevated plasma levels of TP by a large amount.

An elevated risk of erythrocyte degeneration is associated with a high TB serum concentration.[50] There is a deficiency in bile excretion by the liver as a result of the liver damage caused by hepatotoxin (CCl_4), which is manifested in their elevated serum levels.[51] Polygonum persicaria (200 & 400 mg/kg) and silymarin (100 mg/kg) pretreatment greatly reduced serum TB levels to normal control levels, implying increased hepatic capacities.

The isolated compounds' ability to scavenge DPPH radicals is largely determined by their hydrogen donation ability. The DPPH radical scavenging activity assay was used to compare the compounds' radical scavenging behaviours to those of vitamin C, a typical antioxidant.

The scavenging potential of the compounds was determined using their IC_{50} values, which are the effective concentrations at which the DPPH radical is scavenged by 50%. A low IC_{50} value means that a sample has high radical scavenging activity. Polygonum persicaria and Ascorbic acid were shown to have potent free radical scavenging abilities. The use of the DPPH assay method for determining antioxidant activity[52] is now widely suggested. The antioxidant activity of Polygonum persicaria water extract in our in vitro assays showed high antioxidant activity in the DPPH assay. Furthermore, alkaloids, flavonoids, tannins, sterols, and saponins were included in our preliminary phytochemical screening of the Polygonum persicaria sample. The existence of antioxidant and free radical scavenging phytoconstituents such as polyphenol, flavonoids, and saponins, which are believed to have hepatoprotective properties, may be due to the antioxidant activity of the Polygonum persicaria extract.

The body has a mechanism in place to prevent and destroy free radical-induced damage. Many endogenous cancer preventive agent catalysts, such as glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and glutathione S transferase, help to cultivate this. As the balance between ROS generation and cell reinforcement protection is disrupted, oxidative pressure develops, which, over time, deregulates cell capacities, resulting in a variety of fearful situations.[53] Any natural or synthetic compound with cancer-preventive properties can lead to the partial or full acceleration of this type of injury. The first line of defence against free radicals is GSH. Glutathione (GSH) is a non-protein thiol found in living organisms that plays a key role in coordinating the body's antioxidant defence mechanisms.[54] GSH is transformed to glutathione disulfide (GSSG) during oxidative stress, resulting in lipid peroxidation. In rats, CCl4 therapy decreased tissue GSH levels as compared to rats treated with CCl4 and plant extracts. CCl4 treatment resulted in a loss of glutathione from the liver and a reduction of its hepatic content. This lower levels may be attributed to a higher use of GSH in the detoxification process.[55]

One of the most important cellular defence mechanisms against electrophilic xenobiotics and their metabolites is GST. CCl_4 administration reduces GST protein levels over time, and GST can use 4-hydroxy-2-nonenal and malondialdehyde as substrates, according to.[56] GST has been stated to be a mutually beneficial defence against ROS.[57] Glutathione peroxidase is a selenoprotein, with two-thirds of it contained in the cytosol and one-third in the mitochondria (in the liver). Glutathione reductase is concerned with maintaining the cell's GSH level (especially in the reduced structure) by causing a rapid reduction of oxidised glutathione to

the reduced state. It's possible that daily cell reinforcements improve the endogenous cancer preventive agent, which protects against ROS depletion and restores the perfect parity by destroying the receptive organisms. They are gaining immense importance as a result of the integrity of their fundamental position in illness antipathy. The activity of glutathione (GSH), glutathione reductase (GR), glutathione peroxidase (Gpx), glucose-6-phosphate dehydrogenase (G6PDH), and glutathione-S-transferase (GST) enzymes decreases dramatically in the toxic group that obtained CCl4 1.5 ml/kg, most likely attributable to protein inactivation by ROS.[58] These modifications were reversed treatment with Polygonum persicaria and silymarin, though the effect was stronger in the silymarin group than in the Polygonum persicaria group. As a result, taking preventative action is more successful than implementing curative therapy.[59]

The hepatoprotective potential of the Polygonum persicaria extract was also verified by observing the histopathology of the liver. The liver portion of the control animals (Figure 1a) showed normal hepatic cells. After CCl4 administration, histopathological differences were observed (Figure 1b), suggesting liver damage and agreeing with previous findings that CCl4 induces cell death[60], mononuclear cell invasion, steatosis, and hepatocyte damage, and accelerates the mitotic process in the liver.[61] Figures 1d and 1e demonstrate that a dosage-dependent liver portion of Polygonum persicaria extract fully reversed all CCl4-induced deteriorations, with moderate necrotic hepatocytes and a congested central vein Figure 1d at a dose of 200 mg/kg only. These findings suggested that therapy with a higher extract dosage (400 mg/kg) increased the histoarchitecture and well-formed hepatic cord arrangements (Figure 1e). Silymarin therapy protected against the hepatotoxicity caused by CCl_4 administration (Figure 1c). Silymarin kept its usual architecture while sustaining less casualties and providing greater protection than Polygonum persicaria alone. In addition, pre-treatment with silymarin resulted in a significant improvement in serum enzyme levels and oxidative parameters in liver tissue. Photomicrographs specifically show that Polygonum persicaria has hepatoprotective properties.

5. CONCLUSION

With CCl_4-induced liver damage in rats, Polygonum persicaria significantly returned all of the measured parameter values to normal. Polygonum persicaria has phenolic compounds and antioxidant ability, according to the results of this study. We found that the aqueous extract of Polygonum persicaria has high antioxidant properties and an important protective activity

against CCl_4-induced acute hepatotoxicity. Both in vitro and in vivo, the Polygonum persicaria extract showed potent antioxidant activities, and the phenolic compounds in the extract may be responsible for their potent antioxidant properties. The hydroxyl group is thought to play an important role in deciding the antioxidant activities of phenolic compounds. Polygonum persicaria can contain many phenolic compounds with substantial antioxidant activity in vitro and in vivo, based on our findings. The PP's activity is also supported by histopathological research. For pharmaceutical trials, Polygonum persicaria should be regarded as a new source of natural antioxidant.

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7. REFERENCES

1. Recknagel RO. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci., 1983; 33: 401-8.
2. Wendel A, Feuersteins S, Konz KH. Acute paracetamol intoxication of starved mice leads to lipid peroxidation in vivo. Biochem Pharmacol, 1987; 8: 2051-3.
3. Dianzani MU, Muzia G, Biocca ME, Canuto RA. Lipid peroxidation in fatty liver induced by caffeine in rats. Int J Tissue React., 1991; 13: 79-85.
4. Hiroshi A, Toshlharu H, Masahiro H, Shoji A. An alteration in liver microsomal membrane of the rat following paracetamol overdose. J Pharm Pharmacol, 1987; 39: 1047-9.
5. Kodavanti PR, Joshi UM, Young YA, Meydrech EF, Mehendale HM. Protection of hepatotoxic and lethal effects of CCl_4 by partial hepatectomy. Toxicol Pathol, 1989; 17: 494-505.
6. Chan, S. H., Tai, M. H., Li, C. Y., and Chan, J. Y. Reduction in molecular synthesis or enzyme activity of superoxide dismutases and catalase contributes to oxidative stress and neurogenic hypertension in spontaneously hypertensive rats. Free Radic. Biol. Med., 40: 2028–2039. doi: 10.1016/j.freeradbiomed.2006. 01.032.
7. Lv, H., Xiao, Q., Zhou, J., Feng, H., Liu, G., and Ci, X. Licochalcone a upregulates Nrf2 antioxidant pathway and thereby alleviates acetaminophen induced hepatotoxicity. Front. Pharmacol, 2018; 9: 147. doi: 10.3389/fphar.2018.00147.

8. Dunaway, S., Odin, R., Zhou, L., Ji, L., Zhang, Y., and Kadekaro, A. L. Natural antioxidants: multiple mechanisms to protect skin from solar radiation. Front. Pharmacol, 2018; 9: 392. doi: 10.3389/fphar.2018.00392
9. Demirdag K, Bakcecioglu IH, Ozercan IH, Ozden M, Yilmaz S, Kalkan A. Role of L-carnitine in the prevention of acute liver damage induced by carbon tetrachloride in rats. Journal of Gastroenterol Hepatol, 2004; 19: 333-8.
10. Anup S, Shivanandappa T. Hepatoprotective effect of the root extract of Decalepis hamiltonii against carbon tetrachloride-induced oxidative stress in rats. Food Chemistry, 2010; 118(2): 411-7.
11. Smuckler EA. Alcoholic drink: Its production and effects. Fed Process, 1975; 34: 2038-44.
12. World Health Organization (WHO). Regional Office for South-East Asia. Regional action plan for viral hepatitis in South-East Asia: 2016-2021. World Health Organization. Regional Office for South-East Asia. 2017. https://apps. who.int/iris/handle/10665/258735.
13. Lu, Y., Zhao, X. M., Hu, Z., Wang, L., and Li, F. LC-MS-based metabolomics in the study of drug-induced liver injury. Curr. Pharmacol. 2018; Rep. 5: 56–67. doi: 10.1007/s40495-018-0144-3.
14. Liu, Q. T., and Zhong, X. Y. Application of metabolomics in neonatal clinical practice. Zhongguo Dang Dai Er Ke Za Zhi., 2019; 21: 942–948. doi: 10.7499/ j.issn.1008-8830.2019.09.019.
15. Cuykx, M., Rodrigues, R. M., Laukens, K., Vanhaecke, T., and Covaci, A. In vitro assessment of hepatotoxicity by metabolomics: a review. Arch. Toxicol, 2018; 92: 3007–3029. doi: 10.1007/s00204-018-2286-9.
16. Pannala, V. R., Vinnakota, K. C., Rawls, K. D., Estes, S. K., O'Brien, T. P., Printz, R. L., et al. Mechanistic identification of biofluid metabolite changes as markers of acetaminophen-induced liver toxicity in rats. Toxicol. Appl. Pharmacol, 2019; 372: 19–32. doi: 10.1016/j.taap.2019.04.001.
17. An, Z., Li, C., Lv, Y., Li, P., Wu, C., and Liu, L. Metabolomics of hydrazine induced hepatotoxicity in rats for discovering potential biomarkers. Dis. Markers, 2018; 8473161. doi: 10.1155/2018/8473161.
18. Hou K.Z. The dictionary of genera of seed plants Chinese Science Press, Beijing (1982), p. 291.
19. Bhupinder S, Pandey VB, Joshi VK, Gambhir SS. J Ethnopharmacol, 1987; 19: 255- 267.

20. Nizar K, Sanghamitra M, Tiwari MP, Singh PN, Vikas, KJ. Herb Med and Toxicol, 2007; 1: 73-79.
21. Tiwari KP, Kumar P, Masood M. J Ind Chem Soc., 1979; 92: 177.
22. Adinarayana D. Chem Abstr 94: 177071u. Leath Scien, 1980; 27: 268-270.
23. USDA, "GRIN taxonomy for plants, Taxon: Persicaria hydropiper (L.) Delarbre," January 2014, http://www.ars-grin.gov/cgibin/npgs/html/tax search.pl.
24. D. T. Loi, The Glossary of Vietnamese Medicinal Plants and Items, Hanoi Medicine Publishing House, Hanoi, Vietnam, 2000.
25. I.B. Harborne, Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 2nd ed., Chapman and Hall, New York NY, USA, 1973.
26. K. Shimada, K. Fujikawa, K. Yahara, T. Nakamura, Antioxidative properties of xanthin and autooxidation of soybean oil in cyclodextrin emulsion, J. Agric. Food Chem., 1992; 40: 945–948.
27. OECD, (Organisation for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity-Acute Toxic Class method. OECD, 2002; Paris.
28. W. Shuangchan, Y. Yuan, T. Hui, L. Zhike, L. Xiaofei, H. Wei, D. Hong, Carthamus red from Carthamus tinctorius L. exerts antioxidant and hepatoprotective effect against CCl_4-induced liver damage in rats via the Nrf2 pathway, J. Ethnopharmacol, 2013; 148: 570–578.
29. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxalocetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology, 1957; 28(1): 56–63.
30. J. King, The hydrolases-acid and alkaline phosphatases, in: Practical Clinical Enzymology, Nostrand Company Ltd., London, 1965; 191–208.
31. H.T. Malloy, K.A. Evelyn. The determination of bilirubin with the photometric colourimeter, J. Biol. Chem., 1937; 119: 481–490.
32. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagent. J Biol Chem., 1951; 193: 265-75.
33. Tayarani I, Cloez I, Clement M, Bourre JM. Antioxidant enzymes and related free elements in aging brain capillaries and chloroid plexus. J Neurochem, 1989; 53: 817-824.
34. Paglia DE, Valentine WN. Studies on qualitative and quantitative characterization of glutathione peroxidase. J Lab clin Med., 1967; 70: 158-169.

35. Askar MA, Sumathy K, ZaheerBaquer N. Regulation and properties of purified glucose6-phosphate dehydrogenase from rat brain. Indian J Biochem Biophys, 1996; 33: 512- 518.
36. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys, 1959; 82: 70–7.
37. Valeer JD. Liver tissue examination. J Hepatol, 2003; 39: 543-9.
38. Wolf PL. Biochemical diagnosis of liver diseases. Indian J Clin Biochem, 1999; 14: 59-90.
39. Chatterjee TK. Medicinal plants with hepatoprotective properties. In Herbal Options. 3rd ed. Calcutta: Books and Allied (P) Ltd, 2000; 135-7.
40. Kim, B. M. The role of saikosaponins in therapeutic strategies for agerelated diseases. Oxid. Med. Cell. Longev, 2018: 8275256. doi: 10.1155/2018/ 8275256.
41. Suja SR, Latha PG, Pushpangadan P, Rajasekharan S. Evaluation of hepatoprotective effects of Helminthostachys Zeylanica (L.) Hook against carbon tetrachloride induced liver damage in wistar rats. J Ethnopharmacol, 2004; 92: 61-6.
42. Yadav NP, Dixit VK. Hepatoprotective activity of leaves of Kalanchoe pinnata Pers. J Ethnopharmacol, 2003; 86: 197-202.
43. Bishayee A, Sarkar A, Chatterjee M. The hepatoprotective activity of carrot (Daucus carota L) against carbon tetrachloride intoxication in mouse liver. J Ethnopharmacol, 1995; 47: 69-74.
44. Yadav NP, Dixit VK. Hepatoprotective activity of leaves of Kalanchoe pinnata Pers. J Ethnopharmacol, 2003; 86: 197–202.
45. Anusha M, Venkateswarlu M, Prabhakaran V, Taj SS, Kumari BP and Ranganayakulu D: Hepatoprotective activity of aqueous extract of Portulaca oleracea in combination with lycopene in rats. Indian Journal of Pharmacology, 2011; 43: 563-7.
46. Horn HJ: Simplified LD_{50} (or ED_{50}) Calculations. Biometrics, 1956; 12: 311-22.
47. Ramaiah SK. A toxicologist guide to the diagnostic interpretation of hepatic biochemical parameters. Food Chem Toxicol, 2007; 45: 1551–1557.
48. Yang L, Wang C, Ye J, Lib H. Hepatoprotective effects of polyprenols from Ginkgo biloba L. leaves on CCl_4-induced hepatotoxicity in rats. Fitoterapia, 2011; 82: 834–840.
49. Kumar SS, Kumar BR, Mohan GK. Hepatoprotective effect of Trichosanthes cucumerina var cucumerina L. on carbon tetrachloride induced liver damage in rats. J Ethnopharmacol, 2009; 123: 347–350.
50. Singh B, Saxena AK, Chandan BK, Anand KK, Suri OP, SuriSatti KA, et al. Hepatoprotective activity of verbenalin on experimental liver damage in rodents. Fitoterapia, 1998; 69: 134-40.

51. Rao RR. Mechanism of drug induced hepatotoxicity. Indian J Pharmacol, 1973; 5: 313-8.
52. Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol, 2011; 48: 41222.
53. Bandyopadhyay U, Das D, Banerjee KR. Reactive oxygen species: Oxidative damage and pathogenesis. Curr Sci., 1999; 77: 658-66.
54. Gueeri H. Influence on prolonged ethanol intake on the level and turnover of alcohol and aldehyde dehydrogenase and glutathione. Adv Exp Med Biol, 1995; 23: 12–14.
55. Rajagopal SK, Manickam P, Periyasami V, Namasivayam N. Activity of Cassia auriculata leaf extract in rats with alcoholic liver injury. J Nutr Biochem, 2003; 14: 452–8.
56. Sultana R, Sunkesula B, Rajua S, et al. Formation of acetaldehyde adducts of glutathione-S-transferase A3 in the liver of animals administered alcohol chronically. Alcohol, 2005; 35: 57–66.
57. Valko M, Leibfritz D, Moncol J, et al. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol, 2007; 39: 44–84.
58. Tabatabaie T, Floyd RA. Susceptibility of glutathione peroxidase and glutathione reductase to oxidative damage and the protective effect of spin trapping agents. Arch Biochem Biophys, 1994; 314: 112–19.
59. Das SK, Vasudevan DM. Protective effects of silymarin, a milk thistle (Silybium marianum) derivative on ethanol-induced oxidative stress in liver. Indian J Biochem Biophys, 2006; 43: 306-311 [PMID: 17133738].
60. Sun F, Hamagawa E, Tsutsui C, Ono Y, Ogiri Y, Kojo S. Evaluation of oxidative stress during apoptosis and necrosis caused by carbon tetrachloride in rat liver. Biochem Biophys Acta, 2001; 1535: 186–91.
61. Teocharis SE, Margeli AP, Skaltsas SD, Spiliopoulou CA, Koutselinis AS. Induction of metallothionein in the liver of carbon tetrachloride intoxicated rats: an immuno-histochemical study. Toxicol, 2001; 161: 129–38.