Polygonum Persicaria (Linn.) and its Active Principle have a hepatoprotective and antioxidant effect on carbon tetrachloride-induced toxicity in rats

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Abstract

The aim of this analysis was to see whether the aqueous extract of the roots of Polygonum persicaria (PP) and its active principle, Tannic Acid (TA), had a hepatoprotective and antioxidant effect in rats provided Carbon tetrachloride (1.5 ml/kg, i.p). Twenty albino wistar rats were divided into five groups: control, CCl4-induced hepatotoxicity, hepatotoxicity with Polygonum persicaria and Tannic acid, and a normal group given 100 mg/kg silymarin. After 14 days, the rats were sacrificed. Toxicity testing was carried out on 12 rats. They were randomly allocated to one of three groups: control, Polygonum persicaria 200 mg/kg (B.wt), and Tannic acid 200 mg/kg (B.wt). The amounts of liver homogenate enzymes (glutathione peroxidase, glutathione-S-transferase, glucose-6-phosphatase dehydrogenase, and glutathione reductase enzymes) were greatly restored by extracts of PP and TA at the tested concentrations, supporting the biochemical results. Tannic acid, in contrast to Polygonum persicaria, tends to have a greater liver defensive role toward carbon tetrachloride-induced hepatotoxicity, as well as antioxidant properties and mild anticancer activity against cell viability at higher concentrations. The histological alterations in the liver indicated the injury. Polygonum persicaria & its active principle Tannic acid has strong antioxidant properties as well as hepatoprotective effects against CCl4-induced hepatotoxicity, as demonstrated by these observations.

Keywords— Polygonum persicaria, Tannic acid, antioxidant, Hepatoprotective, Carbon tetrachloride, Anti cancerous, Silymarin, & Histopathology.

I. INTRODUCTION

The liver performs a surprising amount of essential roles in the body's assistance, execution, and regulation of homeostasis. It is involved in almost all biochemical pathways leading to growth, disease prevention, supplement transmission, vitality control, and proliferation (Sharma et al., 1991). Sugar, carbohydrate, and fat metabolism, detoxification, bile discharge, and nutritional capacity are also essential functions of the liver. As a consequence, keeping a healthy liver is important for good health and prosperity (Subramonium & Pushpangadan, 1999). The liver's frequent and varying sensitivity to xenobiotics allows it to accumulate contaminants from the intestinal tract, resulting in a variety of hepatic damage that is related to a variety of metabolic functions controlled by the liver. Cell death, infection, immune response, fibrosis, ischemia, and impaired gene expression can lead to liver injury, which may vary from acute infectious diseases to hepatoma (Samina et al., 2015).
Hepatotoxicity signifies liver damage caused by synthetics. Certain restorative agents can harm the organ when taken in large doses or, in any case, when presented inside helpful reaches. Hepatotoxicity can also be triggered by other concoction operators, such as those used in research centres and businesses, as well as common synthetic compounds (e.g., microcystins) and home - grown cures. Hepatotoxins are synthetic compounds that cause liver damage. More than 900 drugs have been related to liver injury, and it is the most commonly known cause for a medication's removal from the market. Synthetics often cause liver damage that presents itself in the form of abnormal liver compound tests. Drug-induced liver injury is responsible for 5% of all medical clinic confirmations and half of all serious liver failures. More than 75% of patients with specific drug reactions need liver transplantation or death (Ostapowicz et al., 2002).

One of the most active hepatotoxins, carbon tetrachloride (CCl₄), is commonly used in science studies to test hepatoprotective agents. The hepatotoxic activity of CCl₄ is primarily attributable to chronic or acute exposure to its active metabolite, the trichloromethyl free radical (CCl₃– and/or CCl₃OO–). Hepatocellular lipid aggregation (steatosis), hepatocellular necrosis, or hepatobiliary disease result from prolonged exposure to hepatotoxic substances. Chronic reactions are commonly believed to induce cirrhotic or neoplastic changes. The cause of carbon tetrachloride-induced liver damage was first suggested as peroxidation. The strongly reactive trichloromethyl and trichloromethyl peroxy free radicals are produced when CCl₄ is reductively dehalogenated by the CYP2E1 enzyme (Rose et al., 2014).

The use of herbal medicines for the prevention and control of chronic liver diseases is now gaining the interest of doctors, pharmaceutical firms, and patients; this change is attributed to herbal medicines' efficacy, lack of side effects, and low cost. Plant-derived drugs have a high propensity for scavenging reactive oxygen and nitrogen species developed by hepatocyte, Kupffer, stellate, and endothelial cells in most hepatic complications (C. Loguerco and Federico, 2003). As a result, plant-derived medications tend to be a very appealing choice for the treatment of hepatic diseases (N. Aghel et al., 2007: A. Sehrawat and Sultana, 2006). In the Siddha method of medicine, the herb Polygonum persicaria Linn. (Syn. Persicaria maculosa) and its active concept (Polygonaceae) are included. The plant and its active ingredient have a wide variety of antibiotic, antibacterial, and anticancer effects. Polygonum persicaria aqueous roots extract and its active concept, tannic acid, show anti-inflammatory, antimicrobial, and anticancer function, according to preliminary pharmacological reports. Polygonum persicaria's fresh roots and active principle have anticancer properties (Duiwiejua et al., 1999). The aim of this research is to look into the hepatoprotective properties, antioxidiant potential, and anti-cancer properties.

II. MATERIAL AND METHODS

2.1 Plant materials and Preparation of the extracts.

The fresh plant material Polygonum persicaria was collected from Lethpora, Pulwama Kashmir near the Jhelum river and was identified 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 the room temperature. The dried plant material were then pulverized into coarse powder in a grinding machine. The plant sample of 500g was extracted in distilled water for a period of 3 days. Solvent from sample was filtered, squeezed off and evaporated off under reduced pressure in a rotary evaporator to obtain crude extract. A voucher specimen was kept in our laboratory for future reference.

2.2 Experimental Animals.

Studies were carried out by using adult albino male rats weighing (130± 10g / 12-16 weeks old) were selected from departmental colony and were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic condition under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given ad libitum. 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). Animals were dealt with and thought about as per the rules suggested by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India.

2.3 Experimental design.

Animals were separated into five groups of six rats each. Group I served as normal received only the vehicle (5% gum acacia; 1 ml/kg; p.o), and Group II as a toxicant control, (received only CCl₄). Group III animals were treated with standard silymarin at an oral dose of 100 mg/kg and group IV received the aqueous extract of Polygonum persicaria at an oral dose of 200 mg/kg and group V received the aqueous extract of Tannic acid at an oral dose of 200 mg/kg, as a fine suspension of 5% aqueous gum acacia. The treatment was continued for 14 days, once daily. On the day of 14 for groups III-V, 30 min
post-dose of extract administration animals received CCl₄ at the dose of 1.5 ml/kg (1:1 of CCl₄ in olive oil i.p). The rats were anesthetized with ether and blood samples were gathered in tubes for biochemical examination by the retro-orbital puncture method. Blood samples were centrifuged for 10 min at 3000 rpm to isolate the serum. After blood assortment, the animals were sacrificed using ether anaesthesia and the liver tissue was reaped for histopathological studies.

2.4 Biochemical assays

Glutathione-S-transferase, glutathione reductase (Tayarani et al., 1989), glucose-6-phosphatase dehydrogenase (Askar et al., 1996), and glutathione peroxidase (Paglia & Valentine, 1972) were among the new liver tissues that were easily treated for the estimation of metabolic enzymatic exercises.

2.5 Antioxidant activity measured by DPPH:

Using the steady radical, the free radical rummaging movement was estimated as far as hydrogen giving or radical scavenging ability (Gupta et al., 2011). A 0.1 mM DPPH solution in ethanol was added to 3ml of concentrate at various concentrations (10-50 g/ml). The absorbance was assessed at 517 nm after thirty minutes. As a control, butylated hydroxy toluene (BHT) was used. Higher free radical scavenging behaviour is shown by the reaction mixture's lower absorbance.

2.6 Statistical analysis

The data were expressed as mean value ± S.E. statistical significance of difference between various treatments were analyzed by Student’s ‘t’ test followed by one-way analysis of variance (ANOVA) according to (Snedecor & Cochran, 1994). P values ≤0.05 were considered as statistically significant.

III. RESULTS

The toxicant effect of carbon tetrachloride and the protective effect of pre-treatment with plant extract and active theory are seen in Table 1. Toxicant induced a significant (P≤0.05) reduction in the activities of the GR and GPx enzymes in the liver. The functions of these enzymes were significantly protected when PP and TA were given orally. When compared to PP therapy, TA showed improved effects, with more than 70% safety in liver GR and GPx function. The results obtained from TA-treated animals were found to be more similar to those obtained from silymarin-treated animals.

When contrast to the test group, there was a significant inhibition of G-6PDH and GST exercises after 14 days of CCl₄ inebriation. The extract and active theory guideline significantly increased the drained enzymatic activities. Although the recoupment with the extract and active theory was unmistakable, 5 days post-treatment with Tannic acid had a notable effect, as evidenced by the highest percent protection up to (70-90 percent) in association with Polygonum persicaria (50-60%). At the 5% mark, analysis of variance revealed a high level of assurance.

### 3.1 Table 1: Effect of therapeutic agents on activities of antioxidant enzymes.

| Parameters          | Control       | CCl₄ | CCl₄+PP | CCl₄+TA | CCl₄+S | F value |
|---------------------|---------------|------|---------|---------|--------|---------|
| GPx (µ mole/min/protein) | 6.11±0.45    | 3.24±0.18* | 4.59±0.28* | 4.84±0.40* | 5.16±0.36* | 10.6*   |
| GR (µ mole/min/protein)   | 4.37±0.26    | 2.50±0.19* | 3.60±0.23* | 3.91±0.35* | 4.12±0.23* | 9.13*   |
| GST (µ mole/min/protein)  | 8.09±0.56    | 3.71±0.29* | 6.11±0.44* | 7.26±0.46* | 7.47±0.56* | 15.6*   |
| G6PDH (µ mole/min/protein)| 10.3±0.55    | 5.42±0.38* | 8.51±0.77* | 9.39±0.54* | 9.81±0.57* | 13.7*   |

Data are mean ± S.E., N = 6, * =Significant at P≤0.05 for ANOVA; **CCl₄ vs C at P≤0.05; *CCl₄+ Therapy vs CCl₄ at P≤0.05
Abbreviations: C= Control; CCl₄= Carbon tetrachloride; S= Silymarin; PP = Polygonum persicaria; TA=Tannic acid, %= Percent protection

Tannic acid and Polygonum persicaria indicated most extreme 65.93±0.05% and 53.63±0.09 DPPH radical scavenging activity at 400 µg/ml concentrations, with ranging concentration from 100-400 µg/ml. Though at a comparative fixation BHT level of restraint was 71% (Table 2). BHT is a food added substance utilized for safeguarding which is liable for kid hyperactivity (Feingold, 1986).
3.2 Table 2: DPPH scavenging activities of extract of Polygonum persicaria and its active principle Tannic acid.

| Concentration (µg/ml) | %Inhibition Polygonum persicaria | %Inhibition Tannic acid | Butylated hydroxyl toluene (BHT) |
|-----------------------|----------------------------------|------------------------|----------------------------------|
| 50                    | 20.96±0.53                       | 32.46±0.07             | 55.35±0.02                       |
| 100                   | 28.07±0.03                       | 33.81±0.12             | 61.04±0.09                       |
| 200                   | 49.06±0.10                       | 51.10±0.08             | 66.81±0.02                       |
| 400                   | 53.63±0.09                       | 65.93±0.05             | 71.03±0.04                       |

The cytotoxic cells were created in a lab setting, away from their usual surroundings. The crude extract reduced cell viability at various concentrations for HeLa (Table 3) and HepG2 (Table 4) cell lines, both of which are very low, and the hindrance was time and portion dependent. In the study of Polygonum persicaria, it was discovered that Tannic acid has greater antiproliferative function. Vitamins, polyphenols, alkaloids, flavonoids, carotenoids, terpenoids, tannins, saponins, enzymes, minerals, and other substances can be responsible for the antiproliferative movement of medicinal plants.

3.3 Table 3: Antiproliferative activity of Tannic acid and Polygonum persicaria against HeLa cell line.

| Concentration µg/ml | 0D Paclitaxel at 580nm | % Cell survival | Tannic acid % Cell survival | Polygonum persicaria % Cell survival |
|---------------------|-------------------------|-----------------|-----------------------------|-------------------------------------|
| 10.85               | 0.162±0.014             | 90.00           | 0.178±0.016                 | 98.87                               |
| 21.70               | 0.148±0.016             | 82.21           | 0.171±0.013                 | 95.00                               |
| 43.40               | 0.132±0.014             | 73.32           | 0.166±0.017                 | 92.21                               |
| 86.80               | 0.122±0.015             | 67.76           | 0.159±0.023                 | 88.32                               |
| 173.60              | 0.115±0.017             | 63.76           | 0.142±0.015                 | 78.87                               |
| 347.20              | 0.180±0.015             | 44.43           | 0.123±0.016                 | 68.32                               |
| 694.40              | 0.048±0.017             | 26.65           | 0.097±0.014                 | 53.87                               |

The inhibition pattern against HeLa cell line at different concentrations. All experiment are triplicates (n=3): mean ± SEM, P>0.05 when test group compared with standard.

3.4 Table 4: Antiproliferative activity of Tannic acid and Polygonum persicaria against HepG2 cell line.

| Concentration µg/ml | 0D Tamoxifen at 580nm | % Cell survival | Tannic acid % Cell survival | Polygonum persicaria % Cell survival |
|---------------------|-----------------------|-----------------|-----------------------------|-------------------------------------|
| 10.85               | 0.177±0.016           | 98.32           | 0.171±0.010                 | 95.00                               |
| 21.70               | 0.159±0.024           | 88.32           | 0.163±0.009                 | 90.54                               |
| 43.40               | 0.145±0.017           | 80.54           | 0.159±0.009                 | 88.32                               |
| 86.80               | 0.130±0.016           | 72.21           | 0.144±0.012                 | 80.00                               |
| 173.60              | 0.129±0.084           | 71.65           | 0.124±0.010                 | 68.87                               |
| 347.20              | 0.118±0.070           | 65.54           | 0.117±0.018                 | 65.00                               |
| 694.40              | 0.085±0.016           | 47.21           | 0.112±0.007                 | 62.21                               |

The inhibition pattern against HepG2 cell line at different concentrations. All experiment are triplicates (n=3): mean ± SEM, P>0.05 when test group compared with standard.
3.5 Histological Studies

Morphological changes towards HeLa cell lines as viewed under the inverted microscope after the post treatment of aqueous extract. (a) Untreated cells, (b) CCl$_4$ treated cells, (c) SLY- Silymarin treated cells, (d) PP- *Polygonum persicaria* treated cells, (e) TA- Tannic acid treated cells.

HeLa cells (a, b, d, e) developed in a logarithmic stage during the treatment with *Polygonum persicaria* and its active principle, resulting in morphological changes, after a pre-treatment of *Polygonum persicaria* and Tannic acid, showing an increased number of modified cells and growth restriction as compared to untreated control cells. Figure (c) depicts the influence of silymarin.

IV. DISCUSSION

In this study, CCl$_4$ was used to induce oxidative stress in the liver, and the resulting hepatic damage was linked to a substantial rise in serum enzymatic and biochemical markers. The development of the active metabolite trichloromethyl radical from CCl$_4$ causes this type of hepatotoxicity [3]. The progressions of CCl$_4$-induced liver damage seen in this study seemed to be similar to those seen in severe viral hepatitis (Venukumar and Latha, 2002). The cytochrome P-450 matrix biotransforms carbon tetrachloride to create the trichloromethyl free radical, which is a commonly used test hepatotoxicant. Which covalently binds to cell layers and organelles to induce lipid peroxidation, disrupt Ca$^{2+}$ homeostasis, and ultimately cause cell death (Recknagel et al., 1989).

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 (Bandyopadhyay et al., 1999). Any natural or synthetic compound with cancer-preventive properties can lead to the partial or full acceleration of this type of injury.

Antioxidant supplements have been shown in some trials to be an effective treatment method for a variety of disorders, including liver inflammation, liver fibrosis, ageing, cancer, and diabetes. Furthermore, reduced GSH activities or content have been identified in a variety of toxicity conditions, suggesting their role in pathogenesis and thus targeting them in the prevention of several toxicity conditions using antioxidants (Meny H et al., 2011). In tissue, GSH acts as a non-enzymatic antioxidant biomolecule. It serves as a substrate for GPx and glutathione S-transferase, as well as removing free oxygen species such as H2O2, superoxide anions, and alkoxyl radicals and maintaining membrane protein thiols (GST).
GSH maintains the body's antioxidant defence system by specifically conjugating with free radicals to defend cell membrane integrity (Bhuwan Chandra et al., 2015). Glutathione peroxidase is a seleno protein with two-thirds of its activity in the cytosol and 33% 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 routine cell reinforcements strengthen 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.

A histological analysis of a liver extract from a CCl₄-treated rat revealed chronic necrosis. When extreme liver damage caused by CCl₄ was significantly decreased by the administration of P.P. (200 mg/kg), T.A. (200 mg/kg), and silymarin (100 mg/kg), as shown by the existence of natural cellular borders, lessened fatty alterations, absence of necrosis, and ballooning degeneration, and widespread lymphocyte infiltration.

V. CONCLUSION

Hepatoprotective function of P.P extract and its active principle TA increases enzymatic and non-enzymatic antioxidant status in relation to GSH material and GR, GPx, G6PDH, and GST activities. The measurement of the scavenging stable DPPH radical is a quick and easy way to assess antioxidant function. The antiradical strength of antioxidant behaviour can be determined using this tool by measuring the decreased absorbance of the DPPH radical, which vanishes after accepting an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus, 2002). Plants in the Polygonaceae family are abundant sources of natural antioxidants and anti-inflammatory agents, thanks to terpenoids, phenols, and flavonoids. Terpenes have been shown to be effective cell reinforcements in a variety of disorders, including liver, renal, neurodegenerative, and coronary disorders, ailments, cancer, and diabetes, as well as in maturing stages (Gonzalez-Burgos & Gomez-Serranillos, 2012). Even though the extract and its active theory differed in their ability to react to and extinguish DPPH radicals, DPPH radical scavenging activity was observed in the tested extract at the chosen portion levels (100-400 µg/ml). However, there were differences in DPPH scavenging activity. Tannic acid scavenging activity was highest at 400 µg/ml (65.93±0.05), followed by 200 µg/ml (51.10±0.08), 100 µg/ml (33.81±0.12), and 50 µg/ml (32.46±0.07). Although the roots fluid of polygonum persicaria aqueous extract showed the most intense scavenging behaviour at 400 µg/ml (53.63±0.09), it was followed by 200 µg/ml (49.06±0.10), 100 µg/ml (28.07±0.03), and 50 µg/ml (20.96±0.09), respectively. When tested at concentrations of 10.85-694.20 µg/ml, the root sections of Polygonum persicaria and its active principle were found to suppress HepG2 and HeLa cancer cells. Cell expansion capacities decreased from low to high component concentrations in the cell practicality experiments. The concentrates demonstrated mild anticancer movement against HeLa and HepG2 cells at a convergence of 694.20 µg/ml; at this concentration, Tannic acid and Polygonum persicaria concentrates have cell suitability of 53.87% and 62.21%, respectively. Furthermore, in the same fixation (694.20 µg/ml), HepG2 treated with paclitaxel and tamoxifen (positive control) demonstrated 26.65% and 47.21% reasonability, respectively, against HeLa. HeLa and HepG2 cells had IC₅₀ values of 27.81 and 33.20 µg/ml, respectively. As compared to standards like paclitaxel and tamoxifen, which are used in cancer chemotherapy, crude concentrate of Tannic acid showed mild anti-proliferative activity against prostate, colorectal, and breast metastatic cell lines (Mamillapalli et al., 2013).

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