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HEPATOPROTECTIVE AND ANTIOXIDANT EFFECT OF POLYGONUM PERSICARIA LINN AND ITS ACTIVE PRINCIPLE ON CARBON TETRACHLORIDE INDUCED TOXICITY IN RATS

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Abstract:

The present study was carried out to observe the hepatoprotective effect and antioxidant activity of the aqueous extract of the roots of *Polygonum persicaria* (PP) and its active principle i.e *Tannic Acid* (TA) in rats treated with carbon tetrachloride (1.5 ml/kg, i.p.). Twenty albino wistar rats were allotted to five groups (control, CCl_4 induced hepatotoxicity and hepatotoxicity with *Polygonum persicaria* and *Tannic acid* and one group acts as a standard treated with silymarin 100 mg/kg. Rats were scarified after 14 days. Toxicity was performed using 12 rats. They were randomly divided into three groups (control and treated with 200 mg/kg (B.wt) of *Polygonum persicaria* & 200 mg/kg (B.wt) of *Tannic acid*. Extract of PP and TA at the tested doses restored the levels of liver homogenate enzymes (glutathione peroxidase, glutathione-S transferase, superoxide dismutase and catalase enzymes significantly and supported the biochemical observations. This study suggests that *Tannic acid* has a more liver protective effect in comparison of *Polygonum persicaria* against carbon tetrachloride- induced hepatotoxicity and possess antioxidant activities and exhibited moderate anticancer activity towards cell viability at higher concentration. Liver injury was confirmed by the histological changes.

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

1. INTRODUCTION

The liver plays a surprising cluster of imperative capacities in the support, execution and controlling homeostasis of the body. It is engaged with practically all the biochemical pathways to development, battle against illness, supplement supply, vitality arrangement and proliferation (Sharma et al., 1991). The significant elements of the liver are sugar, protein and fat digestion, detoxification, discharge of bile and capacity of nutrient. In this way, to keep up a sound liver is a critical factor for the general wellbeing and prosperity (Subramaniam and Pushpangadan, 1999). The continuous and varied exposure of liver to xenobiotics often makes it absorb toxins from the intestinal tract resulting in a variety of hepatic damage which is associated with distortion of many metabolic functions regulated by liver. Liver damage ranges from acute infectious diseases to hepatoma, through cellular death, inflammation, immune response, fibrosis, ischemia, and altered gene expression (Samina Akhter et al., 2015).

Hepatotoxicity suggests synthetic driven liver harm. Certain restorative operators, when taken in overdoses and once in a while in any event, when presented inside helpful reaches, may harm the organ. Other concoction operators, for example, those utilized in research centers and enterprises, common synthetic compounds (e.g., microcystins) and home grown cures can likewise actuate hepatotoxicity. Synthetic compounds that cause liver damage are called hepatotoxins. In excess of 900 medications have been embroiled in causing liver damage and it is the most widely recognized purpose behind a medication to be pulled back from the market. Synthetics frequently cause subclinical damage to liver which shows just as irregular liver compound tests. Drug induced liver damage is answerable for 5% of all medical clinic confirmations and half of all intense liver disappointments. In excess of 75 percent of instances of particular medication responses bring about liver transplantation or passing (Ostapowicz et al., 2002).

Carbon tetrachloride (CCl_4) is one of the most potent hepatotoxins and is widely used in scientific research to evaluate hepatoprotective agents. The hepatotoxic effect of CCl_4 is largely due to its active metabolite, trichloromethyl free radical (CCl_3^- and / or CCl_3OO^-) by chronic or acute vehicles. The regular exposure to hepatotoxic substances causes hepatocellular lipid accumulation (steatosis), hepato-cellular necrosis or hepatobiliary dysfunction5. Cirrhotic or neoplastic changes are usually considered as a result of chronic exposures. Peroxidation was first proposed as mechanism of carbon tetrachloride induced liver injury. The hepatotoxic effect of CCl_4 is a result of its reductive dehalogenation by CYP2E1 enzyme into the highly reactive trichloromethyl and trichloromethyl peroxy free radicals (Rose MH et al., 2014).

Presently, the use of herbal medicines for prevention and control of chronic liver diseases is in the focus of attention for the physicians, pharmaceutical manufacturers, and patients; this shift towards the herbal drugs is due to the effectiveness, less side-effects, and low cost of herbal medicines. Plant-derived drugs are also high potentials for scavenging the reactive oxygen and nitrogen species produced in most of the hepatic complications that involve hepatocyte, Kupffer, stellate, and endothelial cells [C. Loguercio and A. Federico 2003]. Plant-derived drugs, therefore, seem to be very attractive alternative for the healing of hepatic diseases [N. Aghel et al., 2007; A. Sehrawat and S. Sultana, 2006]. The plant *Polygonum persicaria* Linn. (Syn. *Persicaria maculosa*) family (Polygonaceae) and its active principle is utilized in the Siddha arrangement of medication. The plant and its active principle possess a broad spectrum of antibiotic, antibacterial, and anticancer activity. Preliminary pharmacological studies of the plant revealed that the aqueous roots extract of *Polygonum Persicaria* and its active principle i.e tannic acid shows anti-inflammatory, antimicrobial and anticancer activity. The fresh roots of *Polygonum Persicaria and its active principle* have anti cancerous activity (Duwiejua et al., 1999). In this study to explore the hepatoprotective effect, antioxidant potential and anticancerous activities

2. 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 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 were washed with water, cut into pieces and dried in 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±2ºC) 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 CCL_4). Group **III** animals were treated with standard silymarin at an oral dose of 100 mg/kg and group **IV-V** received the aqueous extract of *Polygonum persicaria* at an oral dose of 200 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 CCl_4 at the dose of 1.5 ml/kg (1:1 of CCl_4 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

New tissues of liver were quickly handled for the estimation of metabolic enzymatic exercises included Glutathine-S-transferase, glutathione reductase (Tayarani et al., 1989), glucose-6-phosphatase dehydrogenase (Askar et al., 1996), and glutathione peroxidase (Paglia and Valentine, 1967).

2.5 Antioxidant activity measured by DPPH:

The free radical rummaging movement was estimated as far as hydrogen giving or radical scavenging ability utilizing the steady radical (Gupta et al., 2011).To the 3ml of concentrate at various focuses (10-50 µg/ml), 0.1 mM DPPH solution in ethanol was included. After thirty minutes, the absorbance was estimated at 517 nm. Butylated Hydroxy Toluene (BHT) was utilized as a standard. Lower absorbance of the reaction mixture demonstrates higher free radical scavenging activity.

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 and Cochran,1994). P values ≤0.05 were considered as statistically significant

3. Results

Table 1 depicts That Toxicant impact of carbon tetrachloride and the defensive impact of post treatment with plant extract and active principle. Toxicant caused critical (P≤0.05) hindrance in the exercises of the GR and GPx enzyme in liver (P≤0.05). Oral administration of *PP* and *TA* exhibited a significant protection in the activities of these enzymes. *TA* showed better results when compared with *PP* treatment and showed more than 70% protection in GR and GPx activity of liver. Results obtained from *TA* treated groups were found more close to silymarin treated animals.

A critical inhibition in G-6PDH and GST exercises following 14 days introduction of CCl_4 inebriation, when compared with control group. The extract and active principle guideline freely expanded the drained enzymatic exercises impressively. The recoupment with the extract and active principle was unmistakably apparent yet 5 days post-treatment with Tannic acid showed noteworthy impact as appeared by greatest percent security up to (70-90%) in association with *Polygonum Persicaria* (50-60%). Analysis of variance demonstrated huge assurance at 5% level.

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

Paramters	**Control**	**CCl_4**	**CCl_4+PP**	**CCl_4+TA**	**CCl_4+S**	**F value**
GPx (μ mole/min/protein)	6.12±0.45	3.25±0.18$^{\#}$	4.60±0.28*	4.86±0.40*	5.18±0.36*	10.6$^{@}$
GR (μ mole/min/protein)	4.38±0.26	2.51±0.19$^{\#}$	3.61±0.23*	3.92±0.35*	4.13±0.23*	9.13$^{@}$
GST (μ mole/min/protein)	8.10±0.56	3.72±0.29$^{\#}$	6.11±0.44*	7.27±0.46*	7.48±0.56*	15.6$^{@}$
G6PDH (μ mole/min/protein)	10.4±0.55	5.43±0.38$^{\#}$	8.52±0.77*	9.40±0.54*	9.82±0.57*	13.7$^{@}$

Data are mean ± S.E., N = 6; $^{@}$ =Significant at P≤0.05 for ANOVA; $^{\#}CCl_4$vs C at P≤0.05; *CCl_4+ Therapy vs CCl_4 at P≤0.05 Abbreviations: C= Control; CCl_4= Carbon tetrachloride; S= Silymarin; PP = *Polygonum Persicaria*; TA= *Tannic acid*, %= Percent protection

Tannic acid and *polygonum Persicaria* indicated most extreme 65.94±0.05% and 53.64±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 BF, 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	Tannic acid	Butylated hydroxyl toluene (BHT)
50	20.98±0.53	32.48± 0.07	55.36±0.02
100	28.08±0.03	33.82± 0.12	61.05± 0.09
200	49.07±0.10	51.11± 0.08	66.82±0.02
400	53.64±0.09	65.94± 0.05	71.04±0.04

The cytotoxic cells were developed under controlled conditions, outside of their regular environment.The crude extract diminished the cell feasibility at the diverse concentration for HeLa cell line (Tabel 3) and HepG2 (Table 4) cell line which is very low and the hindrance was time and portion subordinate way. It shows that the Tannic acid has better antiproliferative activity in examination of *polygonum Persicaria*. The different compounds such as vitamins, polyphenols, alkaloids, flavonoids, carotenoids, terpenoids, tannins, saponins, enzymes, minerals etc. so forth may be liable for the antiproliferative movement of such therapeutic 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±0.015	90.00	0.178±0.017	98.88	0.179±0.008	99.44
21.70	0.148±0.017	82.22	0.171±0.013	95.00	0.178±0.014	98.88
43.40	0.132±0.015	73.33	0.166±0.018	92.22	0.177±0.013	98.33
86.80	0.122±0.016	67.77	0.159±0.024	88.33	0.171±0.018	95.00
173.60	0.115±0.018	63.77	0.142±0.016	78.88	0.148±0.017	82.22
347.20	0.180±0.016	44.44	0.123±0.017	68.33	0.135±0.019	75.00
694.40	0.048±0.018	26.66	0.097±0.015	53.88	0.112±0.010	62.22

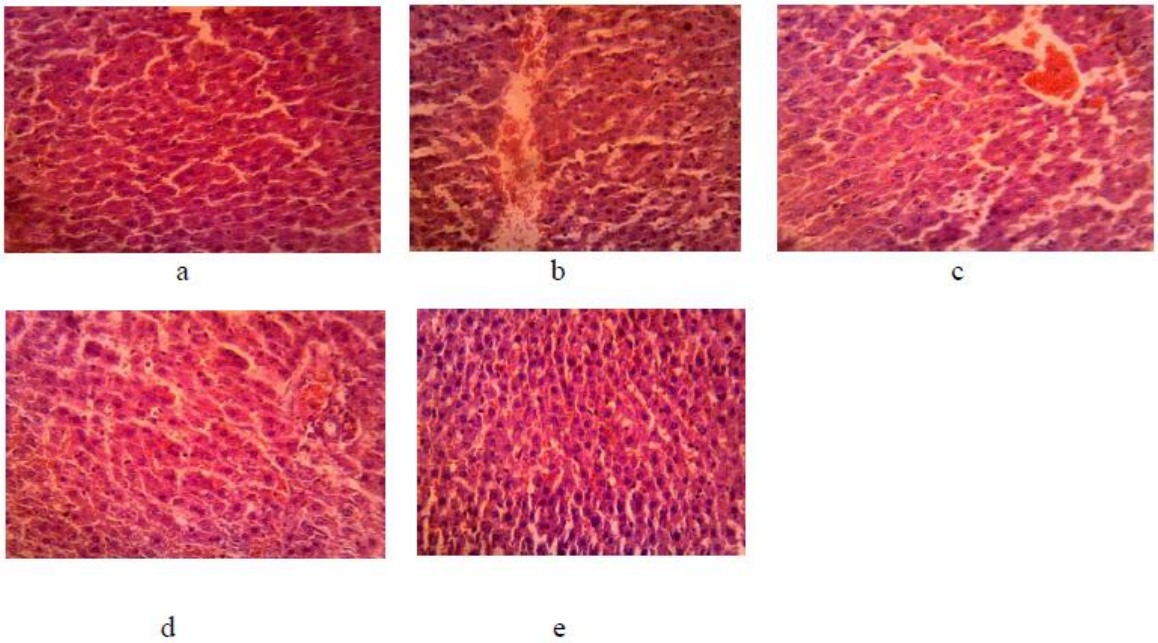
The inhibition pattern against HeLa cell line at different concentrations. All experiment are triplicates (n=3):mean±SEM, P>0.05 when test group compared with standard.

3.4 Table 4: Antiproliferative activity of *Tannic acid* and *Polygonum Persicaria* against HeLa cell line.

Concentration µg/ml	0D Tamoxifen at 580nm	% Cell survival	*Tannic acid*	% Cell survival	*Polygonum persicaria*	% Cell survival
10.85	0.177±0.017	98.33	0.171±0.011	95.00	0.174±0.018	96.66
21.70	0.159±0.025	88.33	0.163±0.010	90.55	0.171±0.011	95.00
43.40	0.145±0.018	80.55	0.159±0.010	88.33	0.166±0.004	92.22
86.80	0.130±0.017	72.22	0.144±0.013	80.00	0.166±0.024	92.22
173.60	0.129±0.085	71.66	0.124±0.011	68.88	0.161±0.024	89.44
347.20	0.118±0.071	65.55	0.117±0.019	65.00	0.147±0.014	81.66
694.40	0.085±0.017	47.22	0.112±0.008	62.22	0.144±0.017	80.00

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) *PP* treated cells (d) *TA* treated cells (e) Silymarin treated cells.

The morphological changes were obtained for HeLa cells **Fig. (a-d)**, which were developing in logarithmic stage all through the treatment with *Polygonum persicaria* and its active principle. After the 48 h post-treatment of *Polygonum persicaria* and *Tannic acid*, **fig (e)** represents the effect of silymarin, an expanded number of adjusted cells and development restraint was seen when compared with the untreated control cells.

4. DISCUSSION

In this research CCl_4 was administrated to cause oxidative stress in liver and the hepatic damage was associated with significant increased level of serum enzymatic and biochemical markers. This sort of hepatotoxicity by CCl_4 is mainly due to the formation of the active metabolite trichloromethyl radical from CCl_4 (Samina Akhter at al., 2015). The progressions related with CCl_4-prompted liver harm of the present examination seemed comparative to that of intense viral hepatitis (Venukumar and Latha, 2002). Carbon tetrachloride, a generally utilized test hepatotoxicant, is biotransformed by the cytochrome P-450 framework to produce the trichloromethyl free radical, which revolve covalently ties to cell layers furthermore, organelles to inspire lipid peroxidation, upset Ca^{2+} homeostasis, and lastly bring about cell demise (Recknagel et al., 1989).

The body has a viable system to avoid and kill the free radical prompted injury. This is cultivated by a lot of endogenous cancer prevention agent catalysts, for example, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and glutathione S transferase. At the point when the harmony between ROS generation and cell reinforcement safeguard is lost, oxidative pressure results, which through a progression of occasions deregulates the cell capacities prompting different fearful conditions (Bandyopadhyay et al., 1999). Any compound, normal or manufactured, with cancer prevention agent properties may contribute towards the fractional or complete acceleration of this kind of injury.

Several studies have demonstrated that antioxidant supplements may be an excellent prevention strategy for many diseases, including liver injury, liver fibrosis, aging, cancer and diabetes. Furthermore, several reports have documented the decreased activities or content of GSH in various toxicity conditions, indicating their implication in pathogenesis and hence targeting them in prevention of many toxicity conditions exploiting antioxidants (Memy H Hassan et al., 2011). The GSH act as non-enzymatic antioxidant bio-molecules present in tissue. It is to remove the free oxygen species, such as H_2O_2, superoxide anions & alkoxy radicals, maintenance of membrane protein thiols, and it acts as a substrate for GPx and glutathione S-transferase (GST). GSH maintaining the body's antioxidant defence mechanism conjugates with free radicals directly to protect the integrity of cell membranes (B.C. Joshi et al., 2015). Glutathione peroxidase is a seleno protein two third (in the liver) is accessible in the cytosol and 33% in the mitochondria. Glutathione reductase is worried about the upkeep of cell level of GSH (particularly in the diminished structure) by affecting quick decrease of oxidized glutathione to diminished state. It might be conceivable that the regular cell reinforcements fortify the endogenous cancer prevention agent guard from ROS desolate and re-establish the ideal parity by killing the receptive species. They are increasing enormous significance by uprightness of their basic job in sickness antipathy.

Additionally, histological examination of liver sample showed chronic necrosis in CCl_4 treated rat. When severe liver injury induced by CCl_4 was markedly reduced by the administration of P.P (200 mg/kg), T.A (200 mg/kg) and silymarin (100 mg/kg), as evident by presence of normal cellular boundaries, lesser fatty changes, absence of necrosis, and ballooning degeneration, broad infiltration of lymphocytes

5. CONCLUSION

Hepatoprotective activity of *P.P* extract and its active principle *TA* increment of enzymatic and non-enzymatic antioxidant status regarding GSH substance and activities of GR, GPx, G6PDH and GST. The determination of the scavenging stable DPPH radical is a very fast method for evaluating the antioxidant activity. Using this method, it is possible to determine the antiradical power of the antioxidant activity by measurement of the reduced absorbance of the DPPH radical which disappears after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule (Matthaus B, 2002). Polygonaceae family plants are most extravagant wellsprings of natural antioxidants, prevention agents, by this gathering of plants having having terpenoids, phenols and flavonoids. Terpenes demonstrated as great cell reinforcements pertinent to oxidative pressure conditions in various illnesses including liver, renal, neurodegenerative and cardiovascular diseases, ailments, malignancy, diabetes just as in maturing forms.(Gonzalez and Gomez 2012). DPPH radical scavenging activity was observed in the tested extract at the chose portion levels (100-400µg/ml), in spite of the fact that the extract and its active principle contrasted in their capacity to respond with and extinguish DPPH radicals. However, there were variations in the scavenging activity of DPPH scavenging activity of *tannic acid* exhibited the maximum scavenging activity at 400 µg/ml (65.94±0.05) followed by at 200 µg/ml (51.11±0.08) at 100 µg/ml (33.82±0.12) at a portion level of 50 µg/ml (32.48±0.07). While roots fluid of aqueous extract of *polygonum Persicaria* demonstrated most extreme scavenging activity at 400 µg/ml (53.64±0.09) trailed by at 200 µg/ml (49.07±0.10) at 100 µg/ml (28.08±0.03) at a portion level of 50 µg/ml (20.98±0.09) respectively. The root parts of of *Polygonum Persicaria* and its active principle were found to express HepG2 and HeLa cancer cell inhibitory action when tried at groupings of 10.85-694.20 µg/ml. In the cell practicality tests, cell expansion capacities diminished from low to high portion concentration.At a convergence of 694.20 µg/ml, the concentrates showed moderate anticancer movement towards HeLa and HepG2 cells, at this focus, concentrate of *Tannic acid* and *Polygonum Persicaria* display cell suitability at 53.88% and 62.22% separately. Additionally, towards HeLa also, HepG2 treated with paclitaxel and tamoxifen (positive control) demonstrated 26.66% and 47.22% reasonability in same fixation (694.20 µg/ml).The IC_{50} values for HeLa and HepG2 cells were 27.82 and 33.21 µg/ml individually Crude concentrate of *Tannic acid* indicated moderate antagonist of proliferative action against prostate, colorectal, and breast metastatic cell lines when contrasted and Standards like paclitaxel and tamoxifen which are utilized in malignancy chemotherapy (Mamillapalli, et al).

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