

## Mechanism of *Polygonum bistorta* and *Zingiber roseum* Against Toxicity

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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 bistorta* (PB) (100 mg/kg) and *Zingiber roseum* (ZR) (100 mg/kg) in rats treated with carbon tetrachloride (0.15 ml/kg, *i.p.*). Extract of PB and ZR at the tested doses restored the levels of liver homogenate enzymes (glutathione peroxidase, glutathione-S-transferase, superoxide dismutase and catalase enzymes significantly). The activities of MTT assay significantly recovered the damage towards normal. This study shows that *Zingiber roseum* has a more liver protective effect in comparison to *Polygonum bistorta* and against carbon tetrachloride-induced hepatotoxicity and possess antioxidant activities and extracts exhibited moderate anticancer activity towards cell viability at higher concentration.

**Keywords:** *Polygonum bistorta*; *Zingiber roseum*; Carbon tetrachloride; Anti cancerous

### Introduction

Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Hepatoprotective effect of some plants like *Spirulina maxima* [1], *Eclipta alba*, *Boehmeria nivea* [2], *Cichorium intybus* [3] and *Picrorhiza kurroo* [4], *Phyllanthus niruri* [5], etc. has been reported. No scientific data have been reported for the hepatoprotective effect of this medicinal plant so far, this study was therefore undertaken to fill the lacuna in this regard.

The plant *Polygonum bistorta* Linn. (Polygonaceae) and *Zingiber roseum* rosc. (Zingiberaceae) is used in the *Siddha* system of medicine. Preliminary pharmacological studies of the plant revealed that the aqueous roots extract of *P. bistorta* and *Z. roseum* shows anti-inflammatory, antimicrobial and anticancer activity. The fresh roots of both plants have anti cancerous activity [6].

### Materials and Methods

#### Plant material and preparation of extract

The plants material was collected by the authorized ayurvedic dealer and was identified by the Botanical Department. Roots extracts of *Polygonum bistorta* and *Zingiber roseum* were shade, dried, coarsely powdered and allowed to heat in distilled water at high temperature of water bath. The inorganic material was precipitated and filtered off. The filtrate was again concentrated in a china dish and dried in vacuum. The yield of the extracts was (10.4 and 10.1%) w/v of powdered aqueous extract, which was stored in refrigerator for further use. The aqueous extract was evaporated and dried in vacuum.

#### Experimental animals

Studies were carried out by using adult albino male rats of *Sprague Dawley* strain (150 ± 10 g) were used for the present investigation. Animals were housed under standard conditions (25 ± 2°C, 60-70% relative humidity and 14 h light and 10 h dark). The rats were fed on standard pellet diet (Pranav Agro Industries, New Delhi) and water *ad libitum*. Animals were treated and cared for in accordance with the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Govt. of India, Ministry of Environment & Forests (Animals Welfare Division), Chennai.

#### Induction of hepatic damage

Liver damage was induced in rats by administering carbon tetrachloride (0.15 ml/kg) in a suspension of olive oil (1:1) *i.p.*, for 21 days [7].

#### Experimental design

Rats were divided into 5 groups of 6 animals each as follows: Group I: served as control and received (olive oil as vehicle only *p.o.*). Group II-V: served as hepatotoxic rats and received carbon tetrachloride (0.15 ml/kg, *i.p.*) with equal volume of olive oil (1:1) for 21 days; Group III: after 21 days CCl<sub>4</sub> administered animals received extract of PB (100 mg/kg *p.o.*) suspended in 2% gum acacia for 5 days. Group IV: after 21 days CCl<sub>4</sub> administered animals received extract of ZR (100 mg/kg *p.o.*) for 5 days. Group V: after 21 days CCl<sub>4</sub> administered animals received silymarin (50 mg/kg *p.o.*) suspended in gum acacia (2%) for 5 days [8].

#### Biochemical assays

Fresh tissues of liver and kidney were immediately processed for the estimation of metabolic enzymatic activities included Glutathione-S-transferase, glutathione reductase [9], glutathione peroxidase [10] and glucose-6-phosphatase dehydrogenase [11].

#### Antiproliferative activity of *Polygonum bistorta* (PB) and *Zingiber roseum* (ZR) on HepG2 cell lines

The MTT assay is a colorimetric assay, which determines the ability of viable cells to convert a soluble tetrazolium salt, MTT [3-(4,5 dimethyl thiazol-2yl)-2,5 diphenyl tetrazolium bromide] into an insoluble formazan precipitate. Tetrazolium salt accept electron from oxidized substrate or appropriate enzyme such as NADH and NADPH.

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Cells were plated into 96-well culture plates. In general, cells should be seeded at densities between 5000 and 10,000 cells per well since they will reach optimal population densities within 48 to 72 h. Experiment was carried out by adding chemicals or biological agents into appropriate well. The volume of tissue culture medium in each well was 0.1 mL, and the medium may contain up to 10% Fetal Bovine Serum. Thaw one vial of MTT solution for each 96-well plate assay. 10  $\mu$ L MTT solution was added to each well. It was mixed by tapping gently on the side of the tray or shakes briefly on an orbital shaker and incubated at 37°C for 4 h. At high cell densities (>100,000 cells per well) the incubation time can be shortened to 2 h. 6. Medium was removed and 200  $\mu$ L DMSO was added into each well to dissolve the formazan by pipetting up and down several times. The absorbance was taken by ELISA plate reader with a test wavelength of 570 nm.

### Statistical analysis

The data were expressed as mean value  $\pm$  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 [12]. P values  $\leq$  0.05 were considered as statistically significant.

### Results

The toxic effect of carbon tetrachloride and the protective effect of post treatment with plant extract and active principle can be seen in Table 1. Toxicant caused significant ( $P \leq 0.05$ ) inhibition in the activities of the GR and GPx enzyme in liver ( $P \leq 0.05$ ). Oral administration of PB and ZR exhibited a significant protection in the activities of these enzymes. ZR showed better results when compared with PB treatment and showed more than 70% protection in GR and GPx activity of liver. Results obtained from ZR treated groups were found more close to silymarin treated animals.

A significant inhibition in G-6PDH and GST activities was seen after 21 days exposure of  $CCl_4$  intoxication, when compared with control group. The extract and active principle independently increased the depleted enzymatic activities considerably. The recouplement with the extract and active principle was clearly evident but 5 days post treatment with *Zingiber roseum* showed significant effect as shown by

maximum percent protection up to (70-90%) in comparison with *P. bistorta* (50-60%). Analysis of variance showed significant protection at 5% level.

The cytotoxic cells were grown under controlled conditions, outside of their natural environment. The antiproliferative activity of the ethyl acetate extract was subjected for MTT assay. In this assay, cell death and cell viability was estimated. The crude extract reduced the cell viability at the different concentration for HepG2 (Table 2) cell line which is very low and the inhibition was time and dose dependent manner. It indicates the *Zingiber roseum* extract has better antiproliferative activity in comparison of *Polygonum bistorta*. The various compounds such as vitamins, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, enzymes, minerals etc. might be responsible for the antiproliferative activity of such medicinal plants.

### Discussion

The changes associated with  $CCl_4$  induced liver damage of the present study appeared similar to that of acute viral hepatitis [13]. Carbon tetrachloride, a widely used experimental hepatotoxicant, is biotransformed by the cytochrome P-450 system to produce the trichloromethyl free radical, which in turn covalently binds to cell membranes and organelles to elicit lipid peroxidation, disturb  $Ca^{2+}$  homeostasis, and finally result in cell death [14].

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase and glutathione S transferase. The loss of balance between ROS production and antioxidant defense results into oxidative stress, which through a series of events which deregulates the cellular functions leading to various pathological conditions [15]. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage.

Glutathione peroxidase is a seleno enzyme two third of which (in liver) is present in the cytosol and one-third in the mitochondria. Glutathione reductase is concerned with the maintenance of cellular level of GSH (especially in the reduced form) by effecting fast reduction

Parameters	Control	$CCl_4$	$CCl_4$ + PB	$CCl_4$ + ZR	$CCl_4$ + S	F value
GR ( $\mu$ mole/min/protein)	4.38 $\pm$ 0.26	2.51 $\pm$ 0.19 <sup>#</sup>	3.61 $\pm$ 0.23*	3.92 $\pm$ 0.35*	4.13 $\pm$ 0.23*	9.13 <sup>@</sup>
GPx ( $\mu$ mole/min/protein)	6.12 $\pm$ 0.45	3.25 $\pm$ 0.18 <sup>#</sup>	4.60 $\pm$ 0.28*	4.86 $\pm$ 0.40*	5.18 $\pm$ 0.36*	10.6 <sup>@</sup>
G6PDH ( $\mu$ mole/min/protein)	10.4 $\pm$ 0.55	5.43 $\pm$ 0.38 <sup>#</sup>	8.52 $\pm$ 0.77*	9.40 $\pm$ 0.54*	9.82 $\pm$ 0.57*	13.7 <sup>@</sup>
GST ( $\mu$ mole/min/protein)	8.10 $\pm$ 0.56	3.72 $\pm$ 0.29 <sup>#</sup>	6.11 $\pm$ 0.44*	7.27 $\pm$ 0.46*	7.48 $\pm$ 0.56*	15.6 <sup>@</sup>

Data are mean  $\pm$  S.E., N = 6; <sup>@</sup> = Significant at  $P \leq 0.05$  for ANOVA; <sup>#</sup> $CCl_4$  vs C at  $P \leq 0.05$ ; \* $CCl_4$  + Therapy vs  $CCl_4$  at  $P \leq 0.05$  Abbreviations: C= Control;  $CCl_4$ = Carbon tetrachloride; S= Silymarin; PB= *Polygonum bistorta*; ZR= *Zingiber roseum*, % = Percent protection

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

Concentration $\mu$ g/ml	OD Tamoxifen at 580 nm	% Cell Survival	<i>Zingiber roseum</i>	% Cell Survival	<i>Polygonum bistorta</i>	% Cell Survival
10.85	0.177 $\pm$ 0.017	98.33	0.171 $\pm$ 0.011	95.00	0.174 $\pm$ 0.018	96.66
21.70	0.159 $\pm$ 0.025	88.33	0.163 $\pm$ 0.010	90.55	0.171 $\pm$ 0.011	95.00
43.40	0.145 $\pm$ 0.018	80.55	0.159 $\pm$ 0.010	88.33	0.166 $\pm$ 0.004	92.22
86.80	0.130 $\pm$ 0.017	72.22	0.144 $\pm$ 0.013	80.00	0.166 $\pm$ 0.090	92.22
173.60	0.129 $\pm$ 0.085	71.66	0.124 $\pm$ 0.011	68.88	0.161 $\pm$ 0.024	89.44
347.20	0.118 $\pm$ 0.071	65.55	0.117 $\pm$ 0.019	65.00	0.147 $\pm$ 0.014	81.66
694.40	0.085 $\pm$ 0.017	47.22	0.112 $\pm$ 0.008	62.22	0.144 $\pm$ 0.017	80.00

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

Table 2: Antiproliferative activities of extracts against HepG2 cell lines.

of oxidized glutathione to reduced state. It may be possible that the natural antioxidants strengthen the endogenous antioxidant defense from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention.

In conclusion, Hepatoprotective action of PB and ZR extract increase of enzymatic and nonenzymatic antioxidant status in terms of GSH contents and activities of GR, GPx, G6PDH and GST. The root parts of two varieties were found to express HepG2 cancer cell inhibitory activity when tested at concentrations of 10.85-694.20 µg/ml. In the cell viability tests, cell proliferation capabilities decreased from low to high dose concentration. At a concentration of 694.20 µg/ml, the extracts exhibited moderate anticancer activity towards HepG2 cells, at this concentration, extract of *Polygonum bistorta* and *Zingiber roseum* exhibit cell viability at 53.88% and 62.22% respectively. Moreover, towards HepG2 treated with paclitaxel and tamoxifen (positive control) showed 22.66% and 47.22% viability in same concentration (694.20 µg/ml). The IC<sub>50</sub> values for HepG2 cells were 27.82 and 33.21 µg/ml respectively Crude extract of rhizome of *Zingiber roseum* showed moderate anti proliferative activity against prostate, colorectal, and breast metastatic cell lines when compared with Standards like paclitaxel and tamoxifen which are used in cancer chemotherapy [16].

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