Research Article

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HEPATOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *POLYGONUM GLABRUM* WILLD. AGAINST ISONIAZID AND RIFAMPIN INDUCED LIVER DAMAGE IN WISTAR RATS

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ABSTRACT

Liver disease is a worldwide problem. Liver is an organ of paramount importance as it plays an essential role in maintaining the biological equilibrium of vertebrates. The conventional drugs used in the treatment of liver diseases viz., corticosteroids, antiviral and immunosuppressive agents are sometimes inadequate and may lead to serious adverse effects. Paradoxically, these may themselves cause hepatic damage. The present study was conducted to evaluate hepatoprotective effect of *Polygonum glabrum* Willd. on Drug induced hepatic damage in Albino Wistar rats. Acute oral toxicity was carried out according to OECD guidelines. The extract was safe up to 2000mg/kg. The administration of extract at 200mg/kg and 400mg/kg showed significant reduction of SGOT, SGPT, ALP, TP, TB and DB as compared to control group. In contrast to this there was considerable increase in the levels of antioxidant parameters like catalase, GSH and LPO in both isoniazid and rifampicin induced hepatotoxicity. There was also improvement in the damage caused to the hepatocytes. From the above findings it was evident that *Polygonum glabrum* has got significant hepatoprotective effect in drug induced hepatotoxicity.

Keywords: Liver, Hepatoprotective, Rifampicin, Isoniazid, drug induced.

INTRODUCTION

In India, numerous medicinal plants are used for liver disorders in traditional system of medicine. Some of these plants are evaluated for hepatoprotective actions against hepatotoxins.¹ However, the readily available hepatoprotective herbal drugs are not sufficiently active to effectively combat severe liver disorders. Therefore, there is a need to develop satisfactory hepatoprotective drugs. Liver has an essential role in maintaining the biological equilibrium of vertebrates. The spectrum of functions includes metabolism and disposition of chemicals to which the organ is exposed directly or indirectly, metabolism of lipids, carbohydrates and proteins, blood coagulation and immunomodulation². The liver plays an important role in maintaining blood glucose levels. It also regulates the circulating blood lipids by the amount of very low density lipoproteins (VLDLs) it secretes. Many of the circulating plasma proteins are synthesized by the liver. In addition, the liver takes up numerous toxic compounds and drugs from the portal circulation. It is well equipped to deal with the metabolism of drugs and toxic substances. The liver also serves as an excretory organ for bile pigments, cholesterol, and drugs. Finally, it performs important endocrine functions.³

Polygonum glabrum Willd. belonging to family polygonaceae is used in the treatment of colic, febrifuge, piles, debility and jaundice. It also possesses anthelmintic⁴ and anti-inflammatory⁵ activities. It has been reported that its antioxidant activity is important in the protection against CCl₄ induced liver toxicity. Therefore the present study is aimed at evaluation of Hepatoprotective effect of *Polygonum glabrum* Willd. on drug induced hepatic damage in Albino Wistar rats.

MATERIALS AND METHODS

Experimental Animals

Wistar albino male rats (180–220 g) were obtained from the central animal house of Sigma Institute of Clinical Research and administration Pvt Ltd Hyderabad. The animals were housed at room temperature (22–28 °C) for 12 h dark and 12 h light cycle and given standard laboratory feed and water ad-libitum. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

Collection of plant material

The whole plant of *Polygonum glabrum* Willd was collected during the month of march 2013 from Tirupati, India. The plant was authenticated by Head, Department of Botany, Sri Venkateshwara University, Tirupathi and voucher specimen of the plant 567/13 was preserved at institute herbarium. Plant was washed, wiped, dried, and subsequently reduced to a coarse powder.

Preparation of plant extract

About 100 g of the powder was separately extracted for 24 h with 75% methanol with intermittent vigorous shaking. The extract was filtered, concentrated with a rotary evaporator and dried over a water bath at 45°C. The residue from the plant parts were used for experimental analysis.

Preliminary Phytochemical Studies

Methanolic extract of the plant of *Polygonum glabrum* Willd was subjected to chemical tests for the identification of chemical constituents.⁶

Acute oral toxicity study

Acute toxicity studies was performed according to OECD-423 guidelines category IV substance (acute toxic class method)⁷. The extract was safe up to 2000 mg/kg.

MATERIAL AND METHODS

Hepatoprotective Activity⁸

Experimental design

Healthy Albino Wistar male rats weighing 180-220g were used. They were randomized into 5 groups of 6 animals each as follows:

Group I: Normal Control, received the vehicle, normal saline (2 ml/kg).

Group II: Negative Control received RIF and INH (100 +50 mg/kg p.o.) at every 72 h for 21 days.

Group III: Received silymarin 50 mg/kg p.o. for 21 days and simultaneously administered RIF and INH (100 +50 mg/kg p.o.) every 72 h.

Group IV: Received plant extract 200 mg/kg p.o for 21 days and simultaneously administered a RIF and INH (100 +50 mg/kg p.o.) every 72 h.

Group V: Received plant extract 400 mg/kg p.o. for 21 days and simultaneously administered RIF and INH (100 +50 mg/kg p.o.) every 72 h.

At the end of all experimental methods blood samples were collected, allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters. The separated serum was used for the estimation of total bilirubin, direct bilirubin, SGOT, SGPT, ALP and total proteins (TP). The animals were sacrificed by administering an excess amount of ether and the livers were removed.

Biochemical estimations

Biochemical parameters i.e aspartate amino transferase (AST)⁹ alanine amino transferase (ALT)¹⁰, alkaline phosphatase (ALP)¹¹, total bilirubin¹² and total protein¹³ were analyzed according to the reported methods. The liver was removed, weighed and morphological changes were observed. A portion of liver was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of protein, superoxide dismutase (SOD)¹⁴, glutathione (GSH)¹⁵ catalase (CAT)¹⁶, and malondialdehyde (MDA)¹⁷.

Histopathological studies

Liver slices were fixed for 12 h in Bouin's solution and were processed for paraffin embedding following standard micro techniques (18). 5µm sections of liver grained with alum haematoxylin and eosin were observed microscopically for histopathological changes. Haematoxyline and eosin were used as staining agents and later microscopic slides of the liver were photographed at the 100x magnification.

Statistical analysis

The data were expressed as mean±SEM values and tested with one way analysis of variance (ANOVA) followed by the Dunnett's multiple comparison test. p<0.05 considered as a significant value.

RESULTS

Results of phytochemical screening

The phytochemical screening of the extract showed the presence of alkaloids, flavanoids, phytosterols and saponins. (Table 1)

Table 1: Phytochemical constituents present in the methanolic extract of *Polygonum glabrum*

Phytoconstituents	Present or Absent
Carbohydrates	+
Glycosides	+
Fixed oils and fats	-
Gums & mucilage	-
Protein & amino acids	+
Saponins	+++
Tannins	+
Phytosterols	++
Flavonoids	++
Alkaloids	+++

Effect of extract of methanolic extract of *Polygonum glabrum* Willd on liver weight

Antitubercular drugs (Rifampicin+Isoniazid) treatment in rats resulted in enlargement of liver which was evident by increase in the weight of wet liver. The groups treated with standard silymarin 50mg/kg (p<0.05) and 200mg/kg extract (non-significant), 400mg/kg (p<0.05) extract showed significant restoration of wet liver weight. (Table 2)

SGOT

Rifampicin+Isoniazid administration resulted in significant (p<0.001) elevation of SGOT levels as compared to normal control group. Treatment with standard drug silymarin 50mg/kg showed significantly (p<0.01) reduction of SGOT level as compared to control group. Administration of extract 200mg/kg and 400mg/kg showed significant reduction of SGOT (p<0.05, p<0.01) as compared to normal control group. (Table 2)

SGPT

Rifampicin+Isoniazid administration resulted in significant (p<0.001) elevation of SGPT levels as compared to normal control group. Treatment with standard drug silymarin 50mg/kg showed significantly (p<0.001) reduction of SGPT level as compared to control group. Administration of extract 200mg/kg and 400mg/kg showed significant reduction of SGPT level (p<0.01, p<0.001) as compared to normal control group. (Table 2)

ALP

Rifampicin+Isoniazid administration resulted in significant (p<0.001) elevation of ALP levels as compared to normal control group. Treatment with standard drug silymarin 50mg/kg showed significantly (p<0.001) reduction of ALP level as compared to control group. Administration of extract 200mg/kg and 400mg/kg showed significant reduction of ALP level (p<0.01, p<0.05) as compared to normal control group. (Table 2)

Total Protein

Rifampicin+Isoniazid administration resulted in significant (p<0.001) elevation of total protein levels as compared to normal group. Treatment with standard drug silymarin 50mg/kg showed significantly (p<0.001) reduction of total protein level as compared to normal control group. Administration of extract 200mg/kg and 400mg/kg showed significant reduction of total bilirubin level (p<0.001, p<0.01) and direct bilirubin level (p<0.0) as compared to normal control group. (Table 3)

Bilirubin

Rifampicin+Isoniazid administration resulted in significant ($p<0.001$) elevation of total and direct bilirubin levels as compared to normal control group. Treatment with standard drug silymarin 50mg/kg showed significantly ($p<0.001$) reduction of total and direct bilirubin level as compared to control group. Administration of extract 200mg/kg and 400mg/kg showed significant reduction of total ($p<0.001$, $p<0.05$) and direct bilirubin level ($p<0.01$, $p<0.05$) as compared to normal control group. (Table 3)

Catalase

RIF and INH treatment caused a significant ($P<0.001$) decrease in the level of catalase, in liver tissue when compared with control group (Table 4). The liver of silymarin 50mg/kg treated animals showed a significant increase ($p<0.001$) in antioxidant enzymes levels compared to INH and RIF treated rats. The treatment of extract at doses of 200 and 400 mg/kg resulted in a significant ($p<0.01$, $p<0.001$) increase of enzymic antioxidants when compared to RIF and INH treated rats.

GSH

RIF and INH treatment caused a significant ($P<0.001$) decrease in the level of GSH in liver tissue when compared with control group (Table 4). The liver of silymarin 50mg/kg treated animals showed a significant increase ($p<0.001$) in antioxidant enzymes levels of GSH as compared to INH and RCIN treated rats. The treatment of extract at doses of 200 and 400 mg/kg resulted in a significant ($p<0.001$, $p<0.001$) increase of enzymic antioxidants of GSH when compared to RIF and INH treated rats.

LPO

RIF and INH treatment caused a significant ($P<0.001$) decrease in the level of LPO in liver tissue when compared with control group (Table 4). The liver of silymarin 50mg/kg treated animals showed a significant increase ($p<0.001$) in antioxidant enzymes levels of LPO as compared to INH and RIF treated rats. The treatment of extract at doses of 200 and 400 mg/kg resulted in a significant ($p<0.05$, $p<0.001$) increase of enzymic antioxidants of LPO when compared to RIF and INH treated rat.

Table 2: Effect of methanolic extract of *Polygonum glabrum* Willd on serum biochemical parameters

Groups	Liver weight g/100 g BW	SGPT U/L	SGOT U/L	ALP U/L
Normal Control(Normal Saline 0.9%)	2.84±0.12	31.86±2.34	51.94±1.64	76.87±1.80
Negative control (Rif and INH)	3.14±0.09 ^a	102.3±2.34a	165.6±1.82a	212.3±3.05a
Standard (Silymarin, 50 mg/kg)	2.77±0.48 *	43.64±2.12***	89.89±1.13**	123.3±2.05***
EPG (200 mg/kg)	3.12±0.15ns	38.59±2.20**	68.02±1.62*	142.7±1.83**
EPG (400 mg/kg)	2.98±0.09*	47.05±2.22***	87.70±1.51**	159.0±2.33*

All the values are expressed as mean±SEM, ns= not significant, N=6, One way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test, * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ as compared to Normal control group. And * $p<0.001$ as compared to Normal group.

Table 3: Effect of methanolic extract of *Polygonum glabrum* Willd on serum total protein and bilirubin

Groups	Total protein (g/dl)	Bilirubin (mg/dl)	
		Total	Direct
Normal control	10.54±0.73	0.18±0.00	0.18±0.02
Negative Control (RIF and INH)	3.29±0.35a	2.10±0.33a	0.54±0.03a
Standard (Silymarin, 50 mg/kg)	7.56±0.34***	0.29±0.02***	0.33±0.01***
MPG (200 mg/kg)	6.92±0.33**	0.33±0.02***	0.30±0.01**
MPG (400 mg/kg)	8.56±0.46***	0.41±0.01*	0.40±0.01*

Table 4: Effect of methanolic extract of *Polygonum glabrum* Willd on *In vivo* antioxidant parameters

Treatment group	<i>In-vivo</i> Antioxidant parameters		
	CATALASE $\mu\text{m}/\text{mg}$ tissue	GSH $\mu\text{m}/\text{mg}$ tissue	LPO μm of $\text{H}_2\text{O}_2/\text{mg}$ tissue
Normal Control	72.33±0.81	3.17±0.05	9.97±0.27
Negative Control (RIF and INH)	30.63±0.84a	1.13±0.06a	24.39±0.59a
Standard Silymarin (50m/kg)	69.70±0.74***	2.42±0.07***	15.64±0.37***
MPG 200mg/kg	46.59±0.91**	1.93±0.01***	15.90±0.65*
MPG 400mg/kg	60.57±0.82***	1.80±0.02***	17.53±0.58***

Results of histopathological studies

Normal Control group: The architecture of liver parenchyma appeared intact. There were seen scattered mononuclear inflammatory cells and epithelioid granulomas with in the parenchyma. Periportal and perivenular infiltration aggregates of mononuclear inflammatory cells were seen. Some of the central veins showed thrombosis.(Slide A)

Negative Control group: The architecture of liver parenchyma appeared partly effaced. There were seen degenerating hepatocytes amidst normal hepatocytes. The parenchyma showed areas of necrosis with mixed inflammatory infiltration. Periportal infiltration by mixed inflammatory cells comprising of lymphocytes, neutrophils and histocytes were seen. (Slide B)

Standard Silymarin group: The architecture of liver parenchyma appeared intact. Focal areas showed degenerating and regenerating hepatocytes. The parenchyma showed aggregates of lymphocytes, macrophages and histocytes. Periportal and perivenular infiltration by mononuclear inflammatory cells comprising of lymphocytes and histocytes were seen (Slide C).

Extract 200mg/kg group: The architecture of liver parenchyma appeared intact. There were seen some regenerating hepatocytes amidst normal hepatocytes. The parenchyma showed aggregates of mononuclear inflammatory cells. Periportal and perivenular infiltration by scattered mononuclear inflammatory cells comprising of lymphocytes and histocytes were seen. bile duct proliferation was seen at some areas (Slide D).

Extract 400mg/kg group: The architecture of liver parenchyma appeared intact. There were seen dilated and congested sinusoids between the hepatocytes. The parenchyma showed few

epithelioid granulomas. Periportal and perivenular infiltration scattered mononuclear inflammatory cells were seen. Some of the central veins showed congestion (Slide E)

A: Normal group

B: Control (RIF and INH)

C: Standard (Silymarin 50mg/kg)

D: Extract 200mg/kg

E: Extract 400mg/kg

Histopathology of Liver

DISCUSSION

The present study was aimed to assess the hepatoprotective activity of methanolic extract of *Polygonum glabrum* Willd. Toxicity studies were conducted in albino rats according to OECD guidelines 423 and was found safe up to the dose level of 2000 mg/kg confirming its non-toxic nature. The hepatoprotective activity was studied in antitubercular drug induced hepatotoxic animal models. The physical parameters such as liver weight and biochemical parameters like SGOT, SGPT, ALP, Total protein and Bilirubin. Antioxidant parameters like catalase, GSH and LPO and histopathology reports of livers were also considered to confirm hepatoprotection.

Antitubercular drug induced hepatotoxicity was significantly prevented by pretreatment with methanolic extract of *Polygonum glabrum* Willd. Decrease in wet liver weight and reduction in biochemical parameters like serum SGOT, SGPT, ALP, Total protein bilirubin and increase in ROS scavenging enzyme activities such as catalase GSH and LPO after treatment with methanolic extract of *Polygonum glabrum* Willd confirmed the hepatoprotective effect of extract. Restoration of hepatic cells with minor fatty changes and absence of necrosis after

treatment with extract indicated satisfactory hepatoprotection. Based on improvement in serum marker enzyme levels, physical parameters, antioxidant parameters and histopathological studies it was concluded that methanolic extract of *Polygonum glabrum*

Willd possesses significant hepatoprotective activity in the doses used¹⁸.

CONCLUSION

In conclusion, the above results demonstrated a potent hepatoprotective action of methanolic extract of *Polygonum glabrum* Willd in Rifampicin and INH-induced oxidative stress and liver toxicity in rats. Such effects can be correlated directly with its ability to reduce lipid peroxidation and enhance the antioxidant defence status. Thus methanolic extract of *Polygonum glabrum* Willd may be used as a safe and effective alternative chemo preventive and protective agent in the management of liver diseases after further studies. Ethnomedicinal plants may provide several bioactive compounds. Hence a detailed study to isolate bioactive compounds from this ethno medicinal plant is required.

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