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Hepatoprotective Activity of *Polygala elongata* against CCl_4-induced Hepatotoxicity in Rats

ANNIE SHIRWAIKAR*, R. PADMA, A. VASANTH KUMAR[1] AND LAKSHMI RAO[2]
Department of Pharmacognosy, College of Pharmaceutical Sciences, Manipal-576 119.
[1]Department of Pharmacology, Kasturba Medical College, Manipal-576 119.
[2]Department of Pathology, Kasturba Medical College, Manipal-576 119.

The ethanolic extract of the plant *Polygala elongata* Klein Ex.Willd (Polygalaceae) was investigated for its hepatoprotective activity in male Wistar rats. In acute toxicity studies, the extract when administered to mice as a single i.p. dose of 1000 mg/kg was found to be non toxic. The extract exhibited significant hepatoprotective activity at 200 mg/kg body weight, which was comparable to the activity exhibited by the reference standard, silymarin in carbon tetrachloride-induced hepatoxicity model. Mangiferin, a xanthone, was the main component isolated from the ethyl methyl ketone fraction of the ethanolic extract. The flavonoid, quercetin-3-O-β-D glucoside was also isolated from the ethyl acetate fraction.

Polygala elongata Klein Ex Wilid (Polygalaceae) (*Mal-Periyananka*) is a small herb or undershrub found in the peninsular India, in Bihar and along the coastal areas of Konkan[1]. A decoction of the leaves is reported to be useful in liver disorders, biliousness and in constipation[1]. A review of literature afforded no information on the hepatoprotective aspects of this plant. In this paper we report the activity of the ethanolic extract of *Polygala elongata* in CCl_4-induced toxicity model in rats[2].

MATERIALS AND METHODS

The plant *Polygala elongata* was collected near the Manipal lake behind the Manipal Junior College, between the months of August and September 1997. It was identified in the Department of Botany, Poorna Prajna College, Udupi-576 101. A voucher specimen has been deposited at the College of Pharmaceutical Sciences, Manipal.

Preparation of plant extract:

The shade-dried powdered plant (1.5 kg) was extracted under reflux for 2.5 h with 95% ethanol. The total ethanolic extract was then evaporated to dryness *in vacuo* (331 g).

***For correspondence**
E-mail: annie.shirwaikar@cops.manipal.edu

The ethyl acetate and the ethyl methyl ketone fractions of the ethanolic extract gave positive tests for flavonoids. Quercetin 3-O-β-D-glucoside (25 mg) and mangiferin (245 mg) were isolated from both these fractions, respectively[3].

Male Wistar rats, weighing about 250 g were used in the present study. They were housed in polypropylene cages in an adequately ventilated room. The rats were fed on standard rat feed pellets supplied by Hindustan Lever Limited, Mumbai and water *ad libitum*. Male mice weighing about 20 to 30 g each were used for acute toxicity studies.

Acute toxicity:

Four groups, having two mice in each, were intraperitoneally administered with ethanolic extracts of *Polygala elongata* at 800, 1000 and 1400 mg/kg doses. The animals were then continuously observed for 1 h, then frequently for 24 h and thereafter once daily for 14 d. During this period, behavioural responses and mortality were recorded.

Hepatoprotective activity:

For determining the hepatoprotective activity animals were divided into 4 groups containing 8 animals each. Group I served as normal control and animals in this group received orally 1 ml propylene glycol per rat (200 g) daily for 5 con-

secutive days. Group 2 served as positive control, received CCl_4, (750 mg/kg/i.p.) followed by 1 ml propylene glycol after 10 min as in group 1. Group 3 was treated with CCl_4, as was done in group 2, followed by administration of total ethanolic extract (200 mg/kg) in 1 ml of propylene glycol. Group 4 animals were treated with CCl_4, as above. Then reference compound silymarin (200 mg/kg) was administered as a suspension in propylene glycol once daily for 5 consecutive days.

Blood samples were withdrawn from all groups on the sixth day by cardiac puncture of nonanaesthetized rats. The serum enzymes, ALT (alanine transferase) and AST (aspartate transferase) were estimated as previously described[4,5]. A small portion of liver (about 0.5 cm was cut from 2 animals from each group and fixed using 4% formalin for histopathological evaluation. The liver sections were stained with haematoxylin and eosin and studied for degenerative and necrotic changes, which were graded as follows. Degeneration was evaluated and graded as 0, when there was no degeneration; as +, when few vacuolated cells per lesion are seen; as ++, when more than 10 cells per lesion are seen; as +++, when 2 rows of vacuolated cells around necrotic zone per lesion are seen. Necrosis was evaluated and graded as 0, when there was no necrosis; as +, when focal necrosis or 1 or 2 cells per lesion are seen; as ++, when focal necrosis of more than 2 cells per lesion are seen; as +++, when massive centrilobular necrosis was seen.

Statistical Analysis:

The results of biochemical estimations have been expressed as mean±S.E (n=8). Data were analysed using the statistical package SPSS/PC + Ver 4.0. One way analysis of Variance (ANOVA)[6,7] followed by Studentised Range Procedure, resulting in the Allowance Value (A) at 95% confidence level was applied for comparison of the means of different groups.

RESULTS AND DISCUSSION

The ethanolic extract did not exhibit any toxic effects upto 1000 mg/kg when administered to mice as a single i.p dose. The LD_{50} of the ethanolic extract was found to be 1200 mg/kg. CCl_4, has been widely used for inducing experimental hepatic damage due to free radical formation during its metabolism by hepatic microsomes[8,9], which in turn causes the peroxidation of cellular membranes leading to the necrosis of hepatocytes. Estimation of serum transaminase levels gives a fairly good idea about the functional state of the liver[10]. The results are reported in Table 1. The total ethanolic extract (group 3) exhibited statistically significant protection against CCl_4-induced increase in the level of serum transaminases in rats, comparable to the pure compound silymarin (group 4). The histopathological studies support the biochemical findings. Hepatotoxicity induced by CCl_4 manifested itself by the 6 d with the livers showing massive degeneration enveloping the not so visible necrotic areas (fig. 2) as compared to the normal control (fig. 1). The liver

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF *POLYGALA ELONGATA* AND SILYMARIN ON CCl_4-INDUCED LIVER DAMAGE IN RATS.

Group (n=8)	AST (I.U./l)	ALT (I.U./l)	ALP (I.U./l)	Histopathology	
				Degeneration	Necrosis
Group 1 (Normal)	203.0±10.23	78.5±5.8	437.3±49.8	-	-
Group 2 (CCl_4)	1013.1±172.9	524.8±108.3	379.7±23.6	++	++
Group 3 (Ethanolic extract)	283.5±92.5	173.5±59.3	109.0±17.26	+	-
Group 4 (Silymarin)	261.5±59.0	110.7±85.00	105.8±9.77	+	+

Values are mean ± SE, n=8, $p<0.001$; CCl_4 was administered at a dose of 0.5 ml/kg i.p for 5 days and ethanolic extract was administered at a dose of 200 mg/kg body weight for 5 consecutive days and silymarin was administered at a dose of 200 mg/kg body weight for 5 consecutive days.

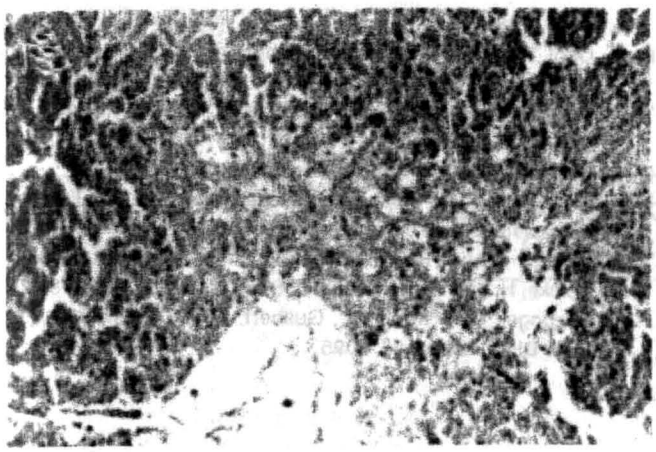

Fig. 1: Liver section of group 1 rat (normal) Haematoxylin and Eosin x 100. Section shows normal structure of hepatic lobules.

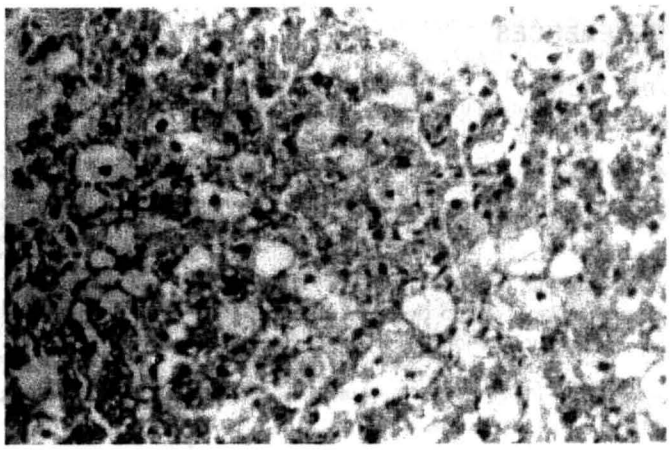

Fig. 2: Liver section of group 2 rat (CCl_4, Control). Haematoxylin and Eosin x 100. Section shows intense fatty degeneration so that necrotic areas are not particularly visible.

sections of rats treated with the ethanolic extract (group 3 fig. 3) were similar to liver sections of group 4 animals (fig. 4) and showed micro vesicular changes with mild congestion and widening of the sinusoids. There was no evidence of necrosis.

Mangiferin rich plants such as *Mangifera indica, Swertia chirata* and *Canscora decussata* are recommended in the Indian system of medicine for the treatment of several immune-deficiency diseases including hepatitis. The bioactivity in mangiferin seems to be mediated by its capacity to provide cellular protection as a systemic antioxidant[11]. Extensive work has been carried out on the hepatoprotective activity of flavonoids such as quercetin, luteolin, apigenin and quercitroside[12]. They are especially useful for the treatment of liver diseases as lipid peroxidation is carried out by flavans. In the light of the above observations, significant protective activity of *Polygala elongata* could be attributed to the flavonoid quercetin and the xanthone mangiferin present in the plant.

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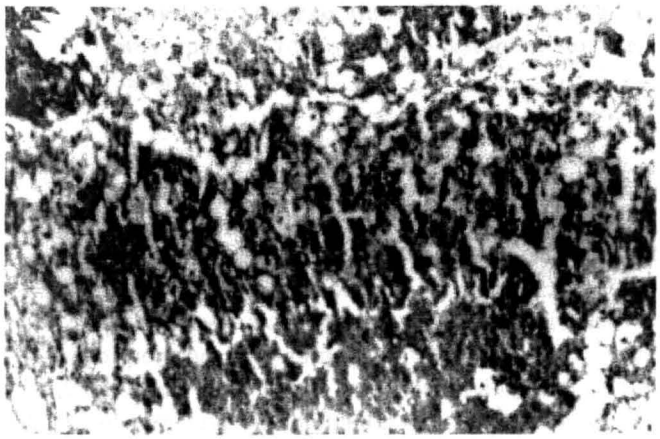

Fig. 3: Liver section of group 3 rat (treated with ethanolic extract of *P. elongata*) Haematoxylin and Eosin x 100. Section shows recovery of hepatic parenchyma, mild congestion and microvesicular changes.

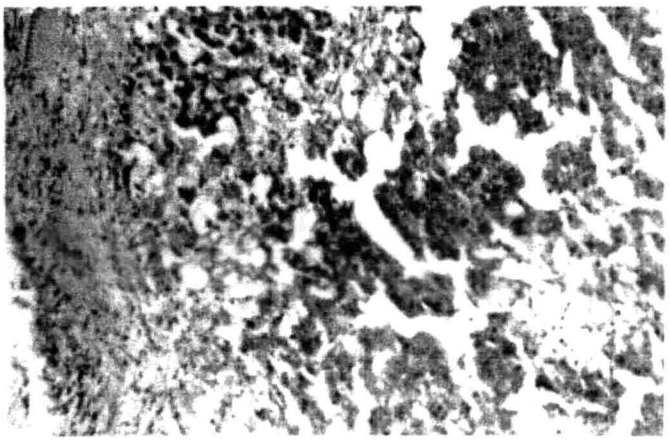

Fig. 4: Liver section of group 4 rat (treated with reference compound silymarin) Haematoxylin and Eosin x 100. Section shows practically complete recovery. Mild congestion of the sinusoids is observed.

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