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Antihepatotoxic Activity of *Swertia chirata* on Paracetamol and Galactosamine Induced Hepatotoxicity in Rats

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The extracts of *Swertia chirata* were evaluated for antihepatotoxic activity using paracetamol and galactosamine models. The methanol extract of the whole plant was found active at a dose of 100 mg/kg i.p. On fractionating this extract into chloroform soluble and butanol soluble fractions, the activity was retained in the chloroform soluble fraction which was most active at a dose level of 25 mg/kg i.p. with overall protection of 81% and 78% against paracetamol and galactosamine, respectively. The butanol soluble fraction, rich in bitter secoiridoids, was devoid of significant activity. The protective effect observed against these two hepatotoxins which are different in their mechanisms of inducing hepatotoxicity, suggests broader and non-specific protection of the liver against these two toxins by non-bitter components of *Swertia chirata.*

Keywords: antihepatotoxic; *Swertia chirata*; paracetamol; D-galactosamine.

INTRODUCTION

The chloroform soluble fraction of *Swertia chirata* was reported as the active antihepatotoxic fraction of the plant against carbon tetrachloride (Karan *et al.*, 1998). Pursuing further investigations, the activity of the plant was assessed against two more toxins of practical importance (a) paracetamol (PcmL) which is a safe antipyretic at therapeutic doses but known to produce liver injury in larger doses in man and experimental animals, (b) D-galactosamine (GalN) which results in acute hepatitis in rats that resembles human viral hepatitis following a single dose of 800 mg/kg.

MATERIALS AND METHODS

Experimental animals. Porton albino rats (100–150 g) of either sex bred in the Central Animal House, Panjab University, Chandigarh were used in the present study. The animals were maintained on standard rat feed (Hind Lever, Bombay or Lipton, Calcutta) and water *ad libitum.*

Chemicals and drugs. The various extracts of *Swertia chirata* were prepared as reported earlier (Karan *et al.*, 1998). The methanol extract was partitioned first with chloroform followed by butanol to obtain a chloroform soluble fraction and a butanol soluble fraction also referred to here as non-bitter and bitter fractions, respectively, on the basis of the nature of the compounds they contained. Paracetamol was procured from Namco National Medicine Company, Amritsar (India). Extracts and paracetamol were administered as a suspension in 50% w/w sucrose solution and D-galactosamine HCl was dissolved in 0.9% NaCl solution prior to administration.

Paracetamol/D-galactosamine model. In each set of experiments the rats were divided in control, toxin and test groups of six animals each. In the paracetamol model (Handa and Sharma, 1990), the animals of the control and of the toxin groups were given sucrose solution when the material under test was given to the test group of animals 48 h, 24 h and 2 h prior to the administration of paracetamol (2 g/kg). Concurrent with the administration of paracetamol to the animals of the test and of the toxin group, the animals in the control group received sucrose solution. The blood was collected from the carotid artery 48 h after the paracetamol administration for biochemical and histopathological studies. Details of the schedule of the paracetamol model are given in Table 1. The schedule of treatment given to various groups of animals under D-galactosamine model was the same as that for paracetamol model (Table 1) except for the substitution of paracetamol with D-galactosamine (800 mg/kg). The extracts and D-galactosamine were administered intraperitoneally and paracetamol was given orally.

Biochemical estimations. Serum transaminases namely, aspartate transaminase (AST) and alanine transaminase (ALT) (Reitman and Frankel, 1957), dehydrogenases, namely sorbitol dehydrogenase (SDH) (Rose and Henderson, 1975) and glutamate dehydrogenase (GIDH) and serum alkaline phosphatase (ALP) (Kind and King, 1954) were estimated by reported methods. Hepatic triglycerides (HTG) were estimated using liver homogenate prepared in 0.9% NaCl solution by the method of Gottfried and Rosenberg (1973). Hepatic glycogen (HGN) was estimated as glucose formed by the

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Table 1. Schedule of treatment of paracetamol/galactosamine model involving pretreatment with the test substance

Group	−48 h	−24 h	−2 h	0 h	+48 h
Control	SS	SS	SS	SS/NS	Biochemical and
PcmL/GalN	SS	SS	SS	PcmL/GalN	histopathological
Test	TS	TS	TS	PcmL/GalN	examination

SS sucrose solution; NS normal saline; TS test substance.
PcmL paracetamol; GalN D-galactosamine.
The '−' sign indicates that the treatment was given prior to the administration of the challenger while the '+' sign indicates the time after the challenger.

Table 2. Effect of various extracts of *Swertia chirata* on rats intoxicated with paracetamol

Group	SSDH (U/L)	SGIDH (U/L)	SGPT (KU)	SGOT (KU)	SALP (IU)	HGN (mg/g.wet wt)	PAV%[a]
Control (A)	2.55 ± 2.18	1.41 ± 0.84	125.1 ± 9.3	90.8 ± 26.8	15.42 ± 3.01	3.08 ± 1.90	0
Paracetamol (B) (2 g/kg p.o.)	69.07 ± 27.22	46.35 ± 24.86	444.7 ± 136.8	277.1 ± 52.7	29.32 ± 7.70	0.40 ± 0.34	100
Methanolle ext. (C) (3 × 100 mg/kg, I.p.)	17.10 ± 10.57 (78.13)	14.25 ± 13.00 (71.43)	213.1 ± 113.1 (72.39)	204.7 ± 33.0 (38.86)	17.10 ± 2.40 (87.91)	3.02 ± 1.66 (97.77)	53
Butanol fraction (D) (3 × 20 mg/kg, I.p.)	23.17 ± 5.63 (69.00)	21.62 ± 16.60 (55.03)	312.2 ± 65.6 (41.43)	271.8 ± 34.7	28.18 ± 9.30	3.73 ± 1.90 (> 100)	61
Chloroform fraction (E) (3 × 100 mg/kg, I.p.)	1.40 ± 0.74 (>100)	1.87 ± 0.44 (98.98)	139.3 ± 10.3 (95.53)	180.8 ± 16.4 (51.70)	16.37 ± 4.17 (93.17)	3.01 ± 0.73 (97.40)	22
F Ratio[b]	25.65	9.61	14.77	28.85	7.97	4.77	
Significant difference between various groups, $p = 0.05$	A & B B & C-E	A & B, D B & C-E	A & B,D B & C-E	A & B-E B & C,E	A & B,D B & C,E	A & B B & C-E	

$n = 6$.
Results: Mean ± SD. Values in parentheses indicate percent protection.
[a] Percentage proportion of abnormal value.
[b] Critical value = 2.69.
The extracts were administered 48 h, 24 h and 2 h prior to paracetamol challenge.

hydrolysis of glycogen in minced liver by the method of Hawk *et al.* (1978).

Histopathological studies. For histopathological examination, 5 μm thick sections of paraffin-embedded liver slices were stained with alum–haematoxylin and eosin following a standard microtechnique described by Galigher and Kozloff (1971) and observed under light microscope.

Statistical analysis. Results of the biochemical estimations are expressed as mean ± SD and the percent proportion of the abnormal value (%PAV) (Perrissoud and Weibel, 1980). The total variation and difference among means were analysed through one-way analysis of variance (ANOVA) and procedure of least significant difference (LSD), respectively (Osol, 1980).

RESULTS AND DISCUSSION

Antihepatotoxic activity in paracetamol model

Oral administration of paracetamol at a dose of 2 g/kg was found to significantly alter the serum levels of SDH, GIDH, AST, ALT, ALP and hepatic glycogen. The level of hepatic triglycerides remained unchanged after paracetamol intoxication, hence were not included in the study. The extent of restoration of the biochemical parameters in the drug treated animals by the toxin compared with the control group reflected the level of activity. The antihepatotoxic activity of the methanol extract (100 mg/kg) was evaluated against paracetamol induced liver damage and was found active (%PAV 53, % protection 47) (Table 2, Group C, Fig. 1). Following fractionation of this extract into a bitter-rich butanol soluble fraction and a non-bitter chloroform soluble fraction, the activity was observed in the latter (Table 3). Different doses (1, 5, 25, 100 and 200 mg/kg) of chloroform soluble fraction were tested and the maximum activity was observed at a dose of 25 mg/kg (%PAV 19, % protection 81) (Table 4, Group E, Fig. 2). On increasing the dose the activity was observed to fall. It was significant to observe that decreasing the dose to 1 mg/kg level still showed 50% overall protection (Table 4, Group C, Fig. 2). A dose of 20 mg/kg of the butanol soluble fraction, which was determined to be the optimum dose against carbon tetrachloride of a number of doses screened (Karan *et al.*, 1998), was found to give overall protection of 39% in the paracetamol model (Table 2, Group D, Fig. 1). The results with all the extracts tested at various dose levels against paracetamol are summarized in Fig. 1 and Fig. 2.

Histopathological examination of the livers of the control group, paracetamol group and the drug treated groups of rats is recorded in photomicrographs in Fig. 3. Liver sections of rats treated with the methanol extract, butanol soluble fraction and chloroform soluble fraction are shown respectively in Fig. 3c, Fig. 3d, Fig. 3e and f.

Figure 3b (paracetamol group) shows gross necrosis of the centrilobular hepatocytes characterized by nuclear

Table 3. Effect of chloroform soluble fraction (50 and 200 mg/kg l.p.) of *Swertia chirata* on rats Intoxicated with paracetamol

Group	SSDH (U/L)	SGIDH (U/L)	SGPT (KU)	SGOT (KU)	SALP (IU)	HGN (mg/g.wet wt)	PAV%[a]
Control (A)	2.87 ± 1.40	1.87 ± 0.32	60.4 ± 9.1	51.5 ± 11.7	14.75 ± 3.09	0.70 ± 0.37	0
Paracetamol (B) (2 g/kg p.o.)	26.42 ± 10.72	26.65 ± 20.90	218.6 ± 89.7	91.2 ± 4.4	21.70 ± 7.81	0.54 ± 0.20	100
Chloroform fraction (C) (3 × 50 mg/kg l.p.)	9.65 ± 1.41 (71.21)	4.13 ± 0.99 (90.88)	48.1 ± 13.6 (>100)	53.6 ± 5.8 (94.79)	15.57 ± 3.88 (88.20)	1.57 ± 1.00 (>100)	34
Chloroform fraction (D) (3 × 200 mg/kg l.p.)	13.18 ± 3.90 (56.22)	9.80 ± 3.94 (68.04)	68.6 ± 18.9 (94.83)	54.3 ± 5.0 (93.10)	13.20 ± 2.73 (>100)	1.81 ± 1.04 (>100)	42
F Ratio[b]	17.54	6.65	17.79	40.33	3.59	3.73	
Significant difference between various groups, $p = 0.05$	A & B,D B & C,D	A & B B & C,D	A & B B & C,D	A & B B & C,D	A & B B & C,D	B & C,D	

$n = 6$.
Results: Mean ± SD. Values in parentheses indicate percent protection.
[a] Percentage proportion of abnormal value.
[b] Critical value = 2.99.
Chloroform soluble fraction was administered 48 h, 24 h and 2 h prior to PcmL challenge.

Table 4. Effect of three lower doses (1, 5, 25 mg/kg i.p.) of chloroform soluble fraction of *Swertia chirata* on rats intoxicated with paracetamol

Group	SSDH (U/L)	SGIDH (U/L)	SGPT (KU)	SGOT (KU)	SALP (IU)	HGN (mg/g.wet wt)	PAV%[a]
Control (A)	2.28 ± 0.37	1.84 ± 0.37	32.1 ± 6.3	90.0 ± 11.9	37.58 ± 15.06	7.47 ± 2.05	0
Paracetamol (B) (2 g/kg p.o.)	27.93 ± 11.25	34.56 ± 10.55	110.24 ± 51.3	157.6 ± 62.8	72.53 ± 20.54	4.21 ± 2.21	100
Chloroform fraction (C) (3×1 mg/kg i.p.)	0.90 ± 0.89 (>100)	2.06 ± 2.21 (90.30)	87.7 ± 45.5	119.5 ± 42.2	48.02 ± 24.32 (96.16)	7.00 ± 2.63 (85.58)	50
Chloroform fraction (D) (3 × 5 mg/kg i.p.)	1.50 ± 1.00 (>100)	2.03 ± 1.17 (99.42)	36.0 ± 17.9 (94.98)	59.9 ± 15.3 (>100)	29.49 ± 9.64 (>100)	4.58 ± 2.40	33
Chloroform fraction (E) (3 × 25 mg/kg i.p.)	1.37 ± 0.73 (>100)	1.28 ± 0.41 (>100)	32.4 ± 9.1 (99.63)	73.0 ± 11.9 (>100)	38.92 ± 10.30 (70.13)	7.90 ± 2.12 (>100)	19
F Ratio[b]	33.51	54.62	7.88	7.36	5.73	2.15	
Significant difference between various groups, $p = 0.05$	A & B B & C-E	A & B B & C-E	A & B,C B & D,E	A & B B & D,E	A & B B & C-E	A & B B & C,E	

$n = 6$.
Results: Mean ± SD. Values in parentheses indicate percent protection.
[a] Percentage Proportion of abnormal value.
[b] Critical value = 2.69.
Chloroform soluble fraction was administered 48 h, 24 h and 2 h prior to PcmL challenge.

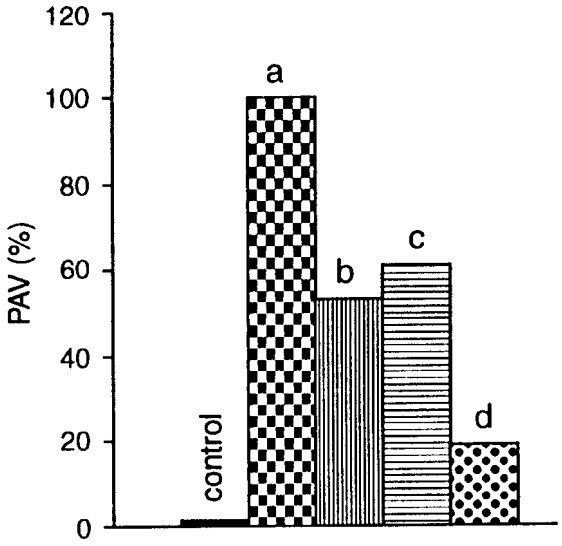

Figure 1. Overall effect of methanol/extract (**b:** 100 mg/kg i.p.), butanol soluble fraction (**c:** 20 mg/kg i.p.) and chloroform soluble fraction (**d:** 25 mg/kg i.p.) of *Swertia chirata* on SSDH, SGIDH, SGPT, SGOT, SALP and HGN in rats intoxicated with paracetamol. The extracts were administered to the rats 48 h, 24 h and 2 h prior to the paracetamol challenge (**a:** 2 g/kg p.o.)

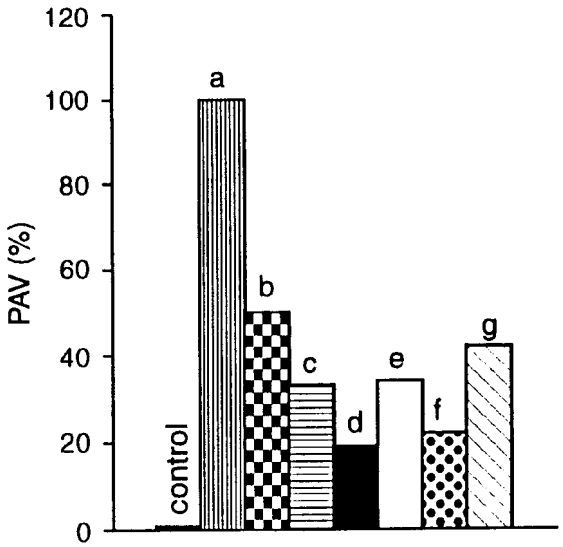

Figure 2. Overall effect of different doses (**b–g:** 1, 5, 25, 50, 100 and 200 mg/kg i.p.) of chloroform soluble fraction of *Swertia chirata* on SSDH, SGIDH, SGPT, SGOT, SALP and HGN in rats, administered 48 h, 24 h and 2 h prior to paracetamol challenge (**a:** 2g/kg p.o.).

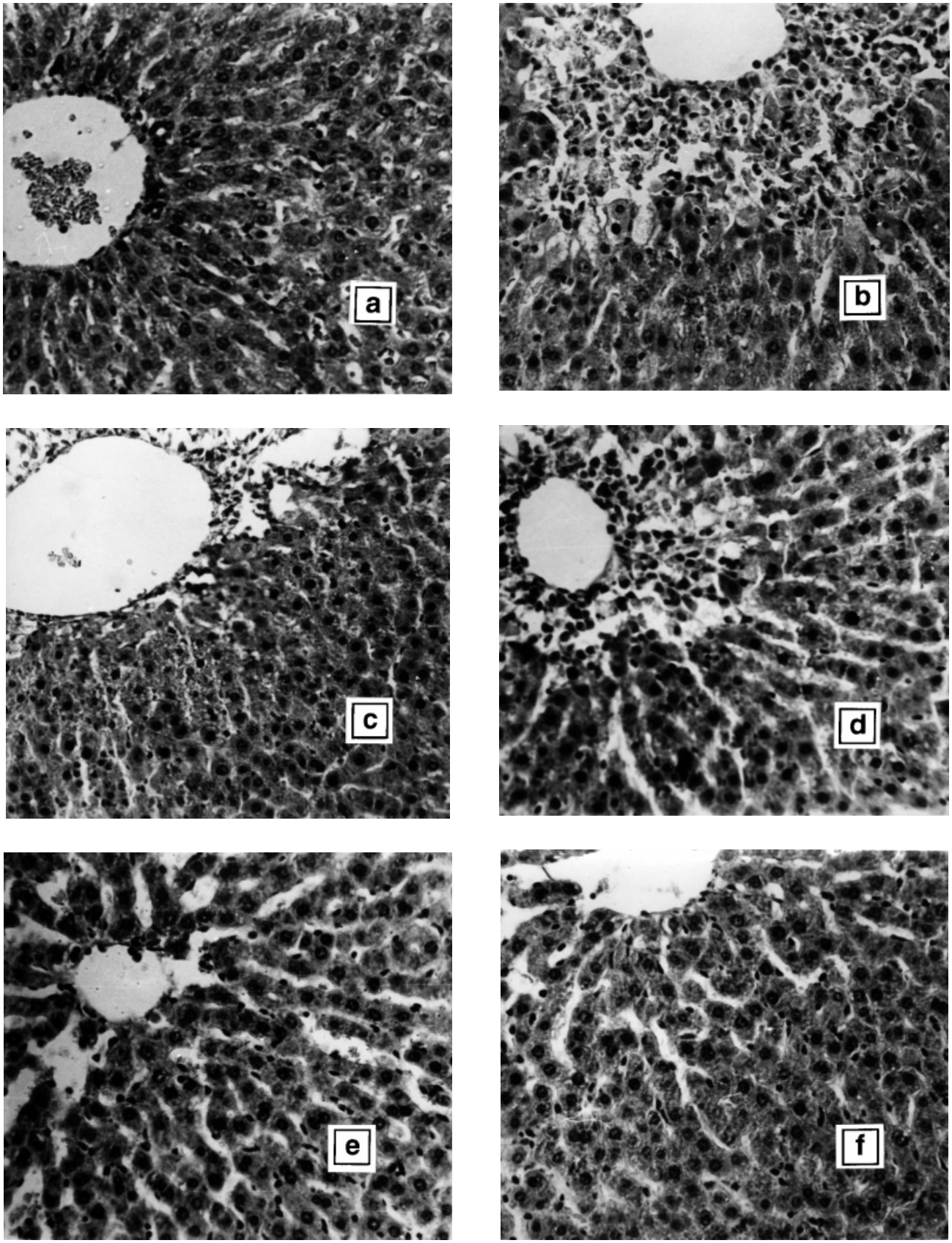

Figure 3. Representative photomicrographs (×330; **1** cm bar = 30.3 μm) of histopathological changes showing effect of *Swertia chirata* on the liver of rats intoxicated with paracetamol. **a:** control; **b:** paracetamol, 2 g/kg p.o.; **c:** methanol extract, 100 mg/kg i.p.; **d:** butanol soluble fraction, 20 mg/kg i.p.; **e** and **f:** chloroform soluble fraction, 100 mg/kg i.p. and 25 mg/kg i.p. respectively.

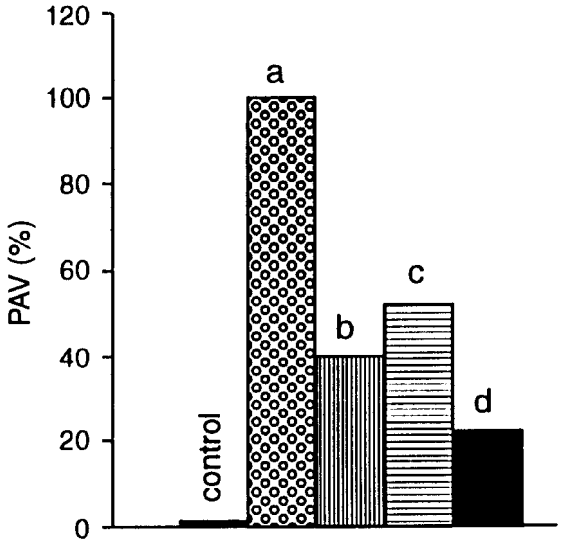

Figure 4. Overall effect of pretreatment with methanol extract (**b:** 100 mg/kg i.p.), butanol soluble fraction (**c:** 20 mg/kg i.p.) and chloroform soluble fraction (**d:** 25 mg/kg i.p.) of *Swertia chirata* on SSDH, SGIDH, SGPT, SGOT, SALP, HTG and HGN in rats intoxicated with galactosamine. The extracts were administered to the rats 48 h, 24 h and 2 h prior to the galactosamine challenge (**a:** 800 mg/kg i.p.).

pyknosis, karyolysis and eosinophilic infiltration caused by paracetamol administration. The liver of rats treated with the methanol extract (Fig. 3c) showed significant recovery. The chloroform soluble fraction at a dose of 100 mg/kg was more active than the methanol extract in reducing the paracetamol toxicity (Fig. 3e). Though it largely restored the liver histoarchitecture, it was the 25 mg/kg dose of the chloroform soluble fraction that did exceedingly well in blocking the paracetamol toxicity (Fig. 3f). The butanol soluble fraction (20 mg/kg) on the other hand suggested no appreciable inhibitory effect on the paracetamol induced toxicity (Fig. 3d) as gross necrosis and mononuclear cell infiltration were observed in the centrilobular region of the liver.

Antihepatotoxic activity in galactosamine model

Though galactosamine is known to differ in the type and extent of the liver damage that it produces, the overall activity profile of *Swertia chirata* in the galactosamine model was found identical to be to that obtained in the carbon tetrachloride and paracetamol models. Only those doses of the different extracts which had exhibited maximum activity in the carbon tetrachloride and paracetamol models were evaluated in the galactosamine model. The methanol extract was active with a PAV percentage of 40 showing 60% overall protection (Table 5, Group C, Fig. 4). The non-bitter chloroform soluble fraction of methanol extract was particularly impressive with a PAV percentage of 22 (overall protection 78%) (Table 5, Group E, Fig. 4). The activity of the butanol soluble bitter fraction was inconsequential, displaying a 48% of overall protection (Table 5, Group D, Fig. 4) but it was a better protection than that observed in the paracetamol model.

Histopathological examination of the liver of the galactosamine treated rats showed focal necrosis especially in the periportal area (Fig. 5b). There was a collapse and condensation of the liver histoarchitecture. Microvesicular fatty change was predominantly observed and there was infiltration of lymphocytes and neutrophils. In contrast, the rats treated with the chloroform soluble fraction (Fig. 5d) retained hepatic architecture quite close to the liver of the control group of rats. With the exception of hepatocytes in the periportal necrotic areas, the hepatocytes by and large retained their shape. The histopathological profile of the rats treated with the methanol extract showed slightly distorted hepatic cords and slight vacuolar necrosis in the periportal cells (Fig. 5c), whereas gross centrilobular necrosis with a broken central canal was observed in rats given the butanol soluble fraction (Fig. 5e), suggesting a lack of activity in this fraction.

Both galactosamine and paracetamol are known hepatotoxic agents which produce fatal hepatic necrosis in toxic doses and have been used extensively in experimental studies (Hinson *et al.*, 1981; Keppler *et*

Table 5. Effect of various extracts of *Swertia chirata* on rats intoxicated with D-galactosamine

Group	SSDH	SGIDH	SGPT	SGOT	SALP	HTG	HGN	PAV%[a]
Control (A)	2.68 ± 0.99	1.67 ± 0.52	120.7 ± 11.6	45.2 ± 16.8	24.13 ± 11.00	7.51 ± 2.24	2.70 ± 1.40	0
Galactosamine (B) (800 mg/kg i.p.)	16.08 ± 4.31	10.57 ± 2.82	205.0 ± 54.8	201.6 ± 45.0	57.76 ± 16.93	16.53 ± 6.95	0.97 ± 0.82	100
Methanol ext. (C) (3×100 mg/kg i.p.)	8.52 ± 3.50 (56.42)	2.17 ± 0.81 (94.38)	133.0 ± 35.0 (85.42)	117.4 ± 10.0 (53.81)	26.15 ± 9.25 (94.00)	8.07 ± 1.16 (93.80)	4.40 ± 1.14 (>100)	40
Butanol fraction (D) (3 × 20 mg/kg i.p.)	8.87 ± 4.27 (53.81)	7.68 ± 1.30 (32.47)	157.9 ± 64.6 (55.91)	191.8 ± 42.6	34.42 ± 6.42 (69.40)	8.67 ± 2.84 (>100)	2.06 ± 0.96	52
Chloroform fraction (E) (3 × 25 mg/kg i.p.)	3.72 ± 0.66 (92.24)	1.90 ± 0.26 (97.53)	110.5 ± 10.7 (>100)	105.3 ± 30.9 (61.59)	21.10 ± 6.99 (>100)	6.82 ± 1.01 (>100)	5.10 ± 1.26 (>100)	22
F Ratio[b]	16.72	47.07	4.92	24.62	11.36	7.46	13.40	
Significant difference between various groups, $p = 0.05$	A & B, D,E B & C-E	A & B,D B & C-E C & D E & D	A & B B & C,E	A & B-E B & C,E C & D D & E	A & B B & C-E	A & B B & C-E	A & B,C,E B & B,C,E C & D D & E	

$n = 6$.
Results: Mean ± SD. Values in parentheses indicate percent protection.
[a] Percentage proportion of abnormal value.
[b] Critical value = 2.69.
Various extracts were administered 48 h, 24 h and 2 h prior to galactosamine challenge.

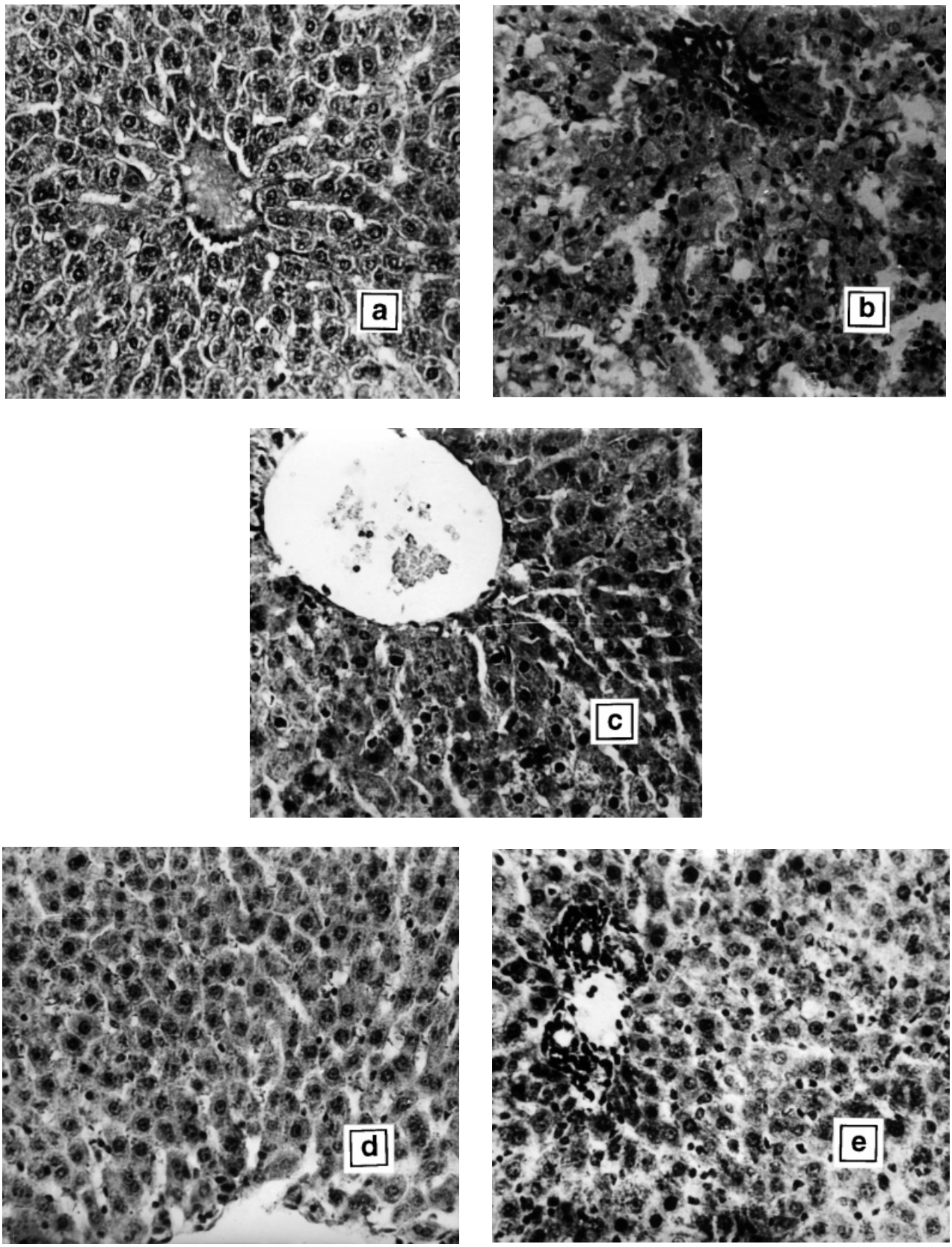

Figure 5. Representative photomicrographs (330×; 1 cm bar = 30.3 μm) of histopathological changes showing effect of *Swertia chirata* on the liver of rats intoxicated with galactosamine. **a:** control; **b:** D-galactosamine, 800 mg/kg i.p. **c:** methanoll extract, 100 mg/kg i.p.; **d:** chloroform soluble fraction, 25 mg/kg i.p.; **e:** butanol soluble fraction, 20 mg/kg i.p.

al., 1968). Galactosamine causes cell injury by depleting uridine nucleotides resulting in inhibition of RNA synthesis leading to necrosis (Farber and EL-Mofty, 1975). Paracetamol is converted into an active metabolic *N*-acetyl-*p*-benzoquinoneimine by the cytochrome-P-450 enzyme system (Savides and Oehme, 1983; Vermeulen *et al.*, 1992). Induction of cytochrome-P-450 or depletion of hepatic glutathione is a prerequisite for the manifestation of paracetamol toxicity (Mitchell *et al.*, 1973). It is evident that the two toxins cause liver injury by different modes and *Swertia chirata* is able to safeguard the liver from both of these toxins, suggesting versatility in its mode of antihepatotoxic activity.

Further, protective mechanisms not specific to galactosamine or paracetamol may be responsible for the antihepatotoxic activity of *Swertia chirata.* The stimulation of hepatic regeneration (Lesch *et al.*, 1970), activation of the function of the reticuloendothelial system (Gruen *et al.*, 1974) or inhibition of protein biosynthesis (Castro *et al.*, 1977; Bachmann *et al.*, 1977) are some of the known mechanisms which can reduce the hepatotoxicity of galactosamine or paracetamol.

CONCLUSIONS

The antihepatotoxic activity of the various extracts of *Swertia chirata* was evaluated in paracetamol and galactosamine induced intoxication in rats which differ in their primary mechanisms of inducing hepatotoxicity. The studies reveal the chloroform soluble fraction (non-bitter in nature) as the most active antihepatotoxic fraction. Further experiments are in progress to identify the phytoconstituents responsible for the antihepatotoxic activity of *Swertia chirata* and to investigate the mechanism of action involved in the antihepatotoxic action. The results are expected to provide a basis for the use of *Swertia chirata* in the indigenous system of medicine for liver disorders.

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