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Efficacy of *Andrgraphis paniculata* (Family: Acanthaceae) methanol extract against paracetamol induced hepathotoxicity in mice

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ABSTRACT

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The 50% methanol extract of *Andrgraphis paniculata* (Syn. Justicia paniculata Burm. F.; Family: Acanthaceae) whole plant except root was evaluated for hepatoprotective activity against over dose of paracetamol (500 mg/kg body wt.) induced hepatotoxicity in albino mice. The mean haemoglobin level and TEC level decreased significantly (P<0.01) in the paracetamol treated group but increased in untreated group from 5th day onwards. Neutrophil, Eosinophil, basophil and monocyte values increased in paracetamol treated group compared to untreated group. The blood glucose level decreased significantly (P<0.01) and the minimum level was found on 11th day in the treated group. The BUN increased significantly (P<0.01) in this group. The serum bilirubin increased significantly (P<0.01) following paracetamol administration. On 15th day, hepatic necrosis with biliary hyperplasia was prominent in the paracetamol treated group. The 50% methanol extract of *Andrgraphis paniculata* seems to have beneficial effect on hepatic toxicity.

Keywords: *Andrgraphis paniculata,* hepatoprotective, hepatotoxic, Justicia paniculata, paracetanol

INTRODUCTION

Andrgraphis paniculata (Syn. justicia paniculata Burn. F.; Family: Acanthaceae) is frequently used hepatoprotective and hepatostimulative agent in the Indian System of Medicine (ISM) against various hepatotoxin which is popularly known as 'Kalmegh'[2]. The effect of diterpines i.e. andrographoide, andrographosides and neoandrographolide from *Andrgraphis paniculata* whole plant was investigated on the hepatocellular antioxidant system in mice and the plant extract increased biliary flow and liver weight in rats[1]. In present study, paracetamol (N-acetyl p-amino phenol, acetaminophen) which is widely used as analgesic and antipyretic drug has been taken as test model to screen anti-hepatotoxic effect of dried 50% methanol extract of *Andrgraphis paniculata* (AP), as over dose of paracetamol (500 mg/kg body wt.) produces liver damage[7,12,13].

MATERIALS AND METHODS

The whole plant of *Andrgraphis paniculata* except root were collected from the Botanical garden of Homoepathic Pharmacopoeia Laboratory (HPL), Ghaziabad, UP. The whole plant was shed dried for one week, powdered mechanically (Sieve 10/44) and stored in air tight container. About 20 g were extracted with the 150 ml 50% methanol for 24 hr by using Soxhlet equipment. The extract was filtered using Whatman filter paper no. 1, and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55^{o}. Dried extract was reconstituted to a final concentration 100 mg/ml and stored in labeled sterile screw-capped bottle in a refrigerator until further use[7]. Healthy, intact Swiss albino mice (*Mus muscularis*) weighing 20-35 g of either sex were used for the study. The animals were housed in poly propylene cages (430x270x150 mm) under standard conditions of light 12:12 hr L:D cycles at 25-28^{o} and 60-80% relative humidity. Feed was given in the form of standard pellets (Lipton, India) with *ad-libitum* clean water. Approval from the institutional ethics committee for the usage of animals was obtained as per the Indian CPCSEA guidelines.

The animals were divided into 4 major groups of 8 mice (4 male and 4 female) each. Group I served as control without any treatment; group II received a single oral dose of paracetamol, as a super saturated solution in 0.85% saline solution @500 mg/kg body weight in order to induce severe liver damage; group III received super saturated solution of paracetamol in 0.85% saline solution @500 mg/kg body weight along with 50% methanol extract of AP (100 mg/100 g/day) for five consecutive days; group IV served as positive control and treated with only 50% methanol extract of AP (100 mg/100g/day) for five consecutive days. All the animals were observed for clinical signs before and after the treatment. Mice were bled under light ether anaesthesia on 0, 5, 11 and 15 day of experiment. Blood samplers were collected from orbital sinus into heparinized tubes. Serum was separated by centrifugation of blood immediately after collection at 3000 rpm for 15 minutes. Haematological parameters like haemoglobin, TEC, TLC and DLC were studied as per the method described earlier[8]. Serum samples were subjected to biochemical parameters viz. Blood glucose, BUN, serum bilirubin, serum protein using standard kits (Qualigens Fine

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Chemicals, Glaxo, India). Results are presented as mean±S.E. and all the data were subjected to student t-test as described[15]. On 5th, 11th and 15th day, 2 mice from each group were sacrificed and necropsied. Representative tissue samples irrespective of presence of gross lesions were collected in 10% formalin. The formalin fixed tissues were processed for histopathological study by conventional paraffin embedding technique[11].

RESULTS AND DISCUSSION

Haemato-biological findings

The results of haematological parameters are presented in table 1. After 24 hours of administration of paracetamol, the animals of group II and III became dull, depressed, showed inappetance with increased respiration and tachycardia. After administration of 50% methanol extract of AP, gradual improvement was seen in group III animals. In group I and IV, the animals were apparently normal. The mean haemoglobin level and TEC level decreased significantly (P<0.01) from 5th day onwards. This is in accordance with the earlier finding[4]. Following administration of AP in group III and IV animals, the mean haemoglobin and TEC levels gradually increased from 5th day onward. In group II animals, the TLC count increased significantly (P<0.01) on 5th and 11th day, whereas lymphocyte count decreased on 5th day, then gradually increased. Neutrophil, eosinophil, basophil and monocyte values increased in group II animals. In group III and IV animals, the mean TLC values decreased, while lymphocyte count increased gradually after 5th day; whereas the neutrophil, eotinophil, basophil and monocyte values decreased, indicating the reduction of hepatic inflammatory changes which was more prominent in gruop III animals.

The results of biochemical parameters are presented in table 2. In group II animals, the blood glucose level decreased significantly (P<0.01) and the minimum level was found on 11th day, whereas, it improved gradually in group IV animals which might be due to gluconeogenesis following AP therapy. The BUN increased significantly (P<0.01) in group II animals, but values decreased gradually after therapy from 5th day and came to normal in group III that was treated with AP. The serum bilirubin increased significantly (P<0.01) following paracetamol administration in group II animals. But the values came to normal in group III animals gradually after therapy with AP, indicating improvement of liver functions[10] and restoration of biliary clearance to normal[6]. No significant changes were seen in serum protein level after therapy.

Histopathological findings

The 5th day result showed mild to moderate degree of congestion in brain of all the animals of group II, but in case of group III, few animals showed mild congestion. In group I and IV, no microscopic changes were noticed through out experiment. The animals of grop II showed mild hyperemia and emphysema of lung on 15th day and diffuse haemorrhage in gastric mucosa on 5th day.

On 5th day, the most prominent changes were found in group II and III animals, which included dilation of hepatic sinusoids, engorgement of central vein with erythrocytes, pyknosis and karyorrhexis of the nuclei along with vacuolar degeneration (Fig. 1), as described earlier in rat[3,5] and mice[12]. In case of group I and IV

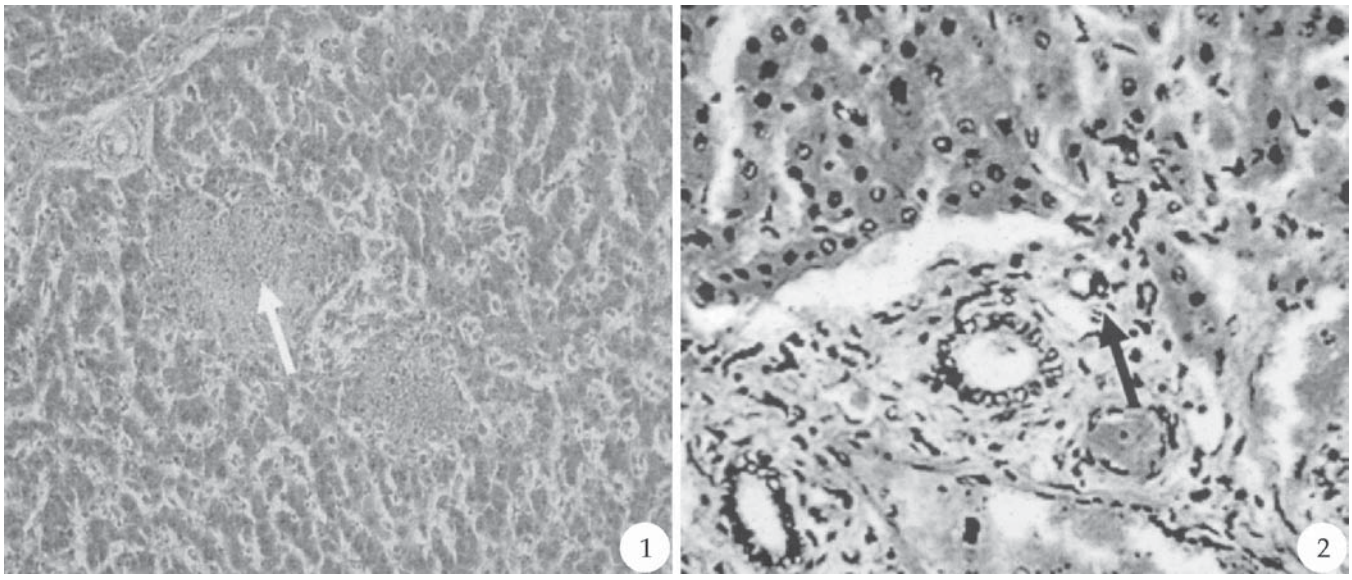

Fig. 1. Hepatic parenchyma showing severe necrosis of hepatic parenchyma (HE x100) **Fig. 2.** Hepatic parenchyma showing biliary epithelial cell proliferation with a tendency to form new bile duct (HE x400).

Table 1. Haematological values in induced hepatotoxicity in mice before and after treatment (Mean±S.E.).

Parameter	Group	0 Day	5th Day	11th Day	15th Day
Hb (gm%)	I	10.25±0.37	10.45±0.33	10.56±0.39	10.72±0.15
	II	10.39±0.21	09.04±0.15**	07.53±0.37**	07.00±0.16**
	III	10.23±0.13	09.26±0.10	09.53±0.44	09.98±0.03
	IV	10.24±0.17	10.38±0.13	10.49±0.26	09.99±0.25
TEC 10^6/µl	I	09.25±0.05	09.36±0.03	09.96±0.02	09.99±0.25
	II	09.78±0.11	07.66±0.10**	07.01±0.05**	06.94±0.22**
	III	07.93±0.72	08.52±0.13	08.19±0.22	08.04±0.21
	IV	07.96±0.93	08.40±0.17	08.37±0.10	08.22±0.09
TLC 10^3/µl	I	07.22±0.34	07.86±0.17	07.49±0.34	08.05±0.37
	II	07.74±0.21	08.77±0.09**	09.91±0.14**	08.64±0.63
	III	07.93±0.72	08.52±0.13	08.19±0.22	08.04±0.21
	IV	07.96±0.93	08.40±0.17	08.37±0.10	08.22±0.09
Lymphocyte (%)	I	58.17±0.31	58.21±0.48	58.36±0.21	58.20±0.30
	II	58.06±0.26	44.31±0.21*	50.61±0.27*	55.11±0.14*
	III	56.16±0.22	45.19±0.23	53.37±0.29**	56.87±0.43**
	IV	58.18±0.17	45.50±0.33	50.50±0.22**	56.55±0.22
Neutrophil (%)	I	32.50±0.28	32.83±0.44	32.90±0.96	34.88±0.97
	II	32.01±0.36	44.67±0.23	40.53±0.52**	36.87±0.80**
	III	32.22±0.33	42.35±0.31	35.32±0.45**	33.30±0.21
	IV	32.66±0.59	43.40±0.21	38.05±0.1**	34.72±0.36**
Eosionophil (%)	I	01.71±0.31	01.78±0.34	01.78±0.89	01.78±0.03
	I	01.81±0.21	05.44±0.23	03.86±0.14**	03.03±0.14
	III	01.79±0.33	03.58±0.12	02.98±0.17	02.15±0.26
	IV	01.86±0.22	04.83±0.44	03.90±0.26	03.24±0.20
Basophil (%)	I	0.21±0.29	01.43±0.24	01.43±0.17	01.90±0.26
	II	0.23±0.45	02.53±0.21	02.02±0.26*	01.19±0.07
	III	0.25±0.38	01.28±0.33	01.14±0.34	01.07±0.19
	IV	0.22±0.26	01.33±0.21	01.22±0.42	01.17±0.13
Monocyte (%)	I	02.55±0.75	02.95±0.48	02.66±0.92	03.27±0.22
	II	02.67±0.35	09.75±0.56	07.88±0.22	05.30±0.31
	III	02.61±0.43	07.55±0.42	05.45±0.21	03.66±0.22*
	IV	02.45±0.49	09.56±0.53	06.33±0.49	05.14±0.049

*Statistically significant at 5% level (<0.05); **Statistically significant at 1% level (<0.01)

Table 2. Biochemical values in induced hepatotoxicity in mice before and after treatment (Mean±S.E.).

Parameter	Group	0 Day	5th Day	11th Day	15th Day
Blood glucose (gm/dl)	I	57.64±0.27	57.74±0.50	58.29±0.32	57.76±.14
	II	58.25±0.11	43.66±0.57	41.35±0.30	44.31±0.26
	III	58.57±0.19	46.10±0.12	47.07±0.47	55.56±0.31
	IV	58.22±0.23	59.15±0.26	30.31±0.14	63.11±0.39
BUN (gm/dl)	I	17.88±0.13	18.06±0.41	18.63±0.56	19.04±0.21
	II	17.23±0.22	35.53±0.19	46.22±0.10	46.410.30
	III	17.26±0.26	30.25±0.31	24.28±0.42	19.95±0.74
	IV	17.91±0.17	31.23±0.24	30.56±0.07	24.00±0.36
Serum bilirubin (mg/dl)	I	0.58±0.02	0.60±0.27	0.59±0.04	0.59±0.01
	II	0.60±0.00	03.66±0.09	03.73±0.04	03.94±0.09
	III	0.61±0.02	02.98±0.08	01.19±0.00	0.79±0.11
	IV	0.64±0.07	03.04±0.06	01.05±0.10	0.86±0.03
Serum protein (gg/dl)	I	05.16±0.04	05.33±0.03	05.39±0.01	05.44±0.21
	II	05.13±0.03	04.01±0.01	03.94±0.03	04.03±0.43
	III	05.33±0.04	05.08±0.10	03.95±0.02	04.16±0.32
	IV	05.44±0.01	04.04±0.033	03.99±0.02	04.24±0.09

*Statistically significant at 5% level (<0.05); **Statistically significant at 1% level (<0.01)

animals, the hepatocytes remained normal. However, on 11th day in group II animals, hepatic necrosis was predominant. The over dose of paracetamol leads of mitochondrial dysfunction followed by hepatic necrosis[10]. The focal areas of haemorrhage were also prominent. Whereas on 11th day, the animals of group III, showed mild degenerative change. On 15th day in group II animals, there was proliferation of biliary epithelial cell with a tendencye to form new bile duct (Fig. 2), which might be due to the toxic effect of paracetamol. In group I and IV animals, the hepatic parenchyma was apparently normal. No visible histopathological changes were recorded on 11th and 15th day.

The renal parenchyma of gruop I and IV animals showed normal glomeruli and renal tubles. Kidneys of group II animals showed congestion and tubular necrosis on 15th day. At some places sloughed tubular epithelium caused bared basement membrane and occlusion of tubular lumen, which confirmed the earlier report[5]. In group III animals on 15th day, renal parenchyma showed normal architecture, mild necrosis with clearly visible tubular lumen[5]. The improved histology of hepatic parenchyma and kidney as observed in group III animals clearly indicated the ability of 50% methanol extract of *Andrgraphis paniculata* to accelerate regeneration of liver cell.

Based on the above results, it can be concluded that 50% methanol extract of *Andrgraphis paniculata* posseses hepatoprotective activity. However, further studies are needed to raionalise the dose and to establish the best formulation for therapeutic use of *Andrgraphis paniculata*.

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