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Research Article

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HEPATOPROTECTIVE ACTIVITY OF *Andrographis paniculata* IN ETHANOL INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

The effect of *Andrographis paniculata* (AP) extract was studied on ethanol induced hepatic damage in rats. Treatment with aqueous extract of *A. Paniculata* (50mg/kg, 100mg/kg, 200mg/kg body weight) was found to protect the rat from hepato-toxic action of ethanol as evidenced by significant reduction in the elevated serum transaminase levels. Histopathological studies show marked reduction in fatty degeneration and centrizonal necrosis in animals receiving different doses of *A. paniculata* along with ethanol as compared to the control group.

Keywords: *Andrographis paniculata*, Ethanol, Hepatoprotective.

INTRODUCTION

Liver disease is a worldwide problem. In the absence of reliable liver[1,2] protective drugs in allopathic medical practices. Herbs play a role in the management of various liver disorders.[3] A number of plants have shown hepatoprotective property. *Andrographis paniculata* (Acanthaceae) is used extensively in the Indian traditional system of medicine[4,5] as hepatoprotective and hepatostimulative agent. The aqueous extract of the leaves of this plant has traditionally been used for treatment of various liver disorders[6] and jaundice.The present study aims to study the influence of the aqueous extract of *Andrographis paniculata* (AP) on serve liver damage leading up to a carcinogen condition.[7,8,9] Therefore this study has been undertaken to evaluate the effect of aqueous extract of *Andrographis Paniculata*(AEAP) on ehanol induced[10,11,12] hepatotoxic in rats.

MATERIALS AND METHODS

Andrographis Paniculata[5] was purchased from Natural Remedies R&D Centre Bangalore.

Drugs and chemicals

Ethanol was obtained from Changshu yangyuan chemical China and biochemical kits was obtained from span diagnostic.[13]

Phytochemical screening

Aqueous extract of *A. paniculata* was screened for various constituents (andrographolide, flavones, lactones) using routine chemical identification methods. Andrographolide is the main constituent and it also active principle of the plant.[4]

Animals and exposure condition

Healthy albino wistar rats (100-120) of male sex[14] were housed under uniform husbandry conditions and given paleted diet (Gold mohur foods and feed Ltd.) and water ad-libitum. The animals were housed at a temperature of 25±2 °C with a 12:12 hrs light and dark cycle was followed.

Study design

Five groups each containing of six rats allotted to different treatment groups. Group 1 (control) was treated with normal saline (10ml/kg) as vehicle only. All other groups received ethanol[15] (24mg/kg p.o, aqueous solution) with group 2 serving as ethanol treated control. After ethanol administration group 3, 4 and 5 also received *A. paniculata* extract 50, 100, 200 kg/kg respectively. The date of 7th, 14th, 21st and 28th day animals were anaesthetized with ether and blood was collected from the retro orbital plexus and serum was separated by centrifugation. The serum was then estimated for ALT, AST, ALP, LDH, Tot. Bil, Dir. Bil. levels[16-18], which has been shown to be elevated by ethanol, rats were killed, liver excised rinsed clean in saline and preserved in 10% formalin for histopathological[19] study (using 5&10 micrometer thick sections stained with haematoxylin eosin(H&E). Results were statistically analysed using students't' test.

Statistical Analysis

All values were expressed as mean ± S.E.M, A 'p' value less than 0.05 was considered statistically significant.

RESULTS

The results for the effect of *Andrographis paniculata* on serum enzymes SGOT, SGPT, LDH, ALP, Total Bilirubin and Direct Bilirubin are shown in Table **1-6**. The administration of ethanol resulted in a marked increase of SGOT, SGPT, LDH, ALP & Bilirubin levels in serum. The toxic effect of ethanol was significantly controlled in the animals treated with aqueous extract of *Andrographis paniculata*.

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Table 1. Effect of extract of *A. paniculata* on serum glutamic oxaloacetic transaminase (SGOT) in albino wistar rats orally administered with Ethanol (20% v/v; 24gm/kg) for 28 days

Treatment Groups	SGOT
	Day 28
I Vehicle control (10 ml/kg)	66.74 ± 2.81
II Intoxicated control, Ethanol (24gm/kg)	96.87 ± 2.87[a]
III Extract of A.paniculata (50 mg/kg)	86.56 ± 4.86
IV Extract of A.paniculata (100 mg/kg)	76.32 ± 2.91[b]
V Extract of A.paniculata (200 mg/kg)	73.96 ± 4.59[b]

Values are expressed as mean ± SEM; n=6
[a] *p ≤ 0.05 Ethanol control Vs Vehicle control.*
[b] *p ≤ 0.05 Extract of A.paniculata Vs Ethanol control.*

Table 2. Effect of extract of *A. paniculata* on serum glutamic pyruvic transaminase (SGPT) in albino wistar rats orally administered with Ethanol (20% v/v; 24gm/kg) for 28 days

Treatment Groups	SGPT
	Day 28
I Vehicle control (10 ml/kg)	23.28 ± 1.39
II Intoxicated control, Ethanol (24gm/kg)	30.47 ±1.21[a]
III Extract of A.paniculata (50 mg/kg)	27.70 ± 0.77
IV Extract of A.paniculata (100 mg/kg)	23.72 ± 4.11[b]
V Extract of A.paniculata (200 mg/kg)	24.38 ± 1.83[b]

Values are expressed as mean ± SEM; n=6
[a] *p ≤ 0.05 Ethanol control Vs Vehicle control.*
[b] *p ≤ 0.05 Extract of A.paniculata Vs Ethanol control.*

Table 3. Effect of extract of *A. paniculata* on lactate dehydrogenase (LDH) in albino Wistar rats orally administered with Ethanol (20% v/v; 24gm/kg) for 28 days

Treatment Groups	LDH
	Day 28
I Vehicle control (10 ml/kg)	1180.21 ± 130.22
II Intoxicated control, Ethanol (24gm/kg)	2000.84 ± 55.10[a]
III Extract of A.paniculata (50 mg/kg)	1645.75 ± 116.12
IV Extract of A.paniculata (100 mg/kg)	1422.66 ± 89.12[b]
V Extract of A.paniculata (200 mg/kg)	1315.79 ± 136.78[b]

Values are expressed as mean ± SEM; n=6
[a] *p ≤ 0.05 Ethanol control Vs Vehicle control.*
[b] *p ≤ 0.05 Extract of A.paniculata Vs Ethanol control.*

Table 4. Effect of extract of *A. paniculata* on alkaline phosphatase (ALP) in albino Wistar rats orally administered with Ethanol (20% v/v; 24gm/kg) for 28 days

Treatment Groups	ALP
	Day 28
I Vehicle control (10 ml/kg)	172.55 ± 13.57
II Intoxicated control, Ethanol (24gm/kg)	333.14 ± 7.45[a]
III Extract of A.paniculata (50 mg/kg)	256.74 ± 23.51
IV Extract of A.paniculata (100 mg/kg)	202.53 ± 18.01[b]
V Extract of A.paniculata (200 mg/kg)	197.52 ± 20.07[b]

Values are expressed as mean ± SEM; n=6
[a] p ≤ 0.05 Ethanol control Vs Vehicle control
[b] p ≤ 0.05 Extract of A.paniculata Vs Ethanol control

Table 5. Effect of extract of *A. paniculata* on total bilirubin in albino Wistar rats orally administered with Ethanol (20% v/v; 24gm/kg) for 28 days

Treatment Groups	Total bilirubin
	Day 28
I Vehicle control (10 ml/kg)	0.118 ± 0.03
II Intoxicated control, Ethanol (24gm/kg)	0.413 ± 0.13[a]
III Extract of A.paniculata (50 mg/kg)	0.187 ± 0.04[b]
IV Extract of A.paniculata (100 mg/kg)	0.151 ± 0.015[b]
V Extract of A.paniculata (200 mg/kg)	0.143 ± 0.08[b]

Values are expressed as mean ± SEM; n=6
[a] *p ≤ 0.05 Ethanol control Vs Vehicle control.*
[b] *p ≤ 0.05 Extract of A.paniculata Vs Ethanol control.*

Table 6. Effect of extract of *A. paniculata* on direct bilirubin in albino Wistar rats orally administered with Ethanol (20% v/v; 24gm/kg) for 28 days

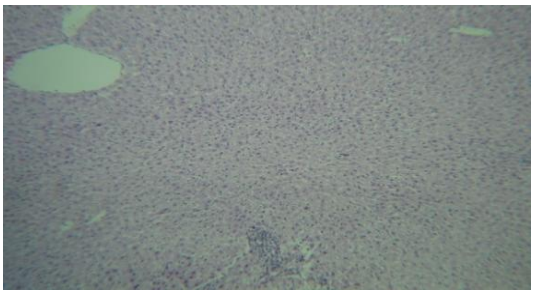
Treatment Groups	Direct bilirubin
	Day 28
I Vehicle control (10 ml/kg)	0.086 ± 0.015
II Intoxicated control, Ethanol (24gm/kg)	0.278 ± 0.016[a]
III Extract of A.paniculata (50 mg/kg)	0.155 ± 0.041
IV Extract of A.paniculata (100 mg/kg)	0.096 ± 0.016[b]
V Extract of A.paniculata (200 mg/kg)	0.092 ± 0.007[b]

Values are expressed as mean ± SEM; n=6
[a] *p ≤ 0.05 Ethanol control Vs Vehicle control.*
[b] *p ≤ 0.05 Extract of A.paniculata Vs Ethanol control.*

Statistical Analysis:
All values were expressed as mean ± S.E.M. A 'p' value less than 0.05 was considered statistically significant.

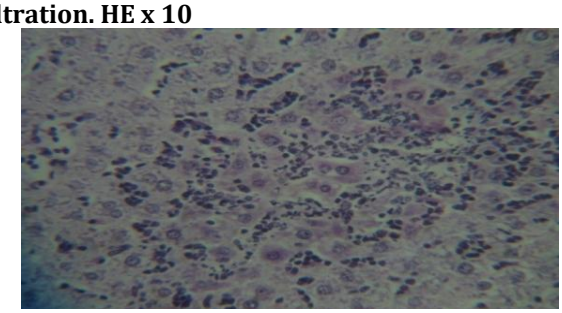
Histopathological Examination
Histopathological profile of liver sections of control group showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Figure **1**). Group II animals exhibited disarrangement of normal hepatic cells with intense centrilobular necrosis, vacuolization of cytoplasm and fatty degeneration (Figure **2**). The liver sections of the rats treated with aqueous extract of *Andrographis paniculata* followed by ethanol intoxication showed a sign of protection as it was evident by the absence of necrosis and vacuoles (Figure **3**, **4** & **5**).

Figure 1. Control rat liver section revealing normal hepatic parenchyma with a central vein at the top corner. HE x 5



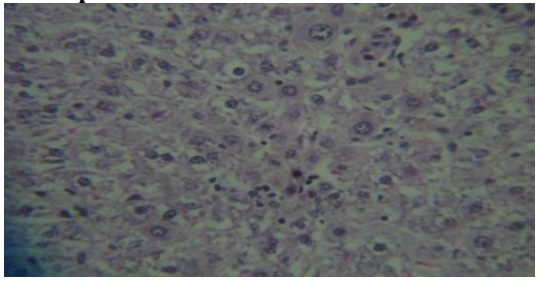
(Normal control 10ml/kg normal saline)

Figure 2. A focus of intense necrotic hepatitis in the diseased control revealing nuclear pyknosis, karyolysis/karyorhexis and intense cellular infiltration. HE x 10



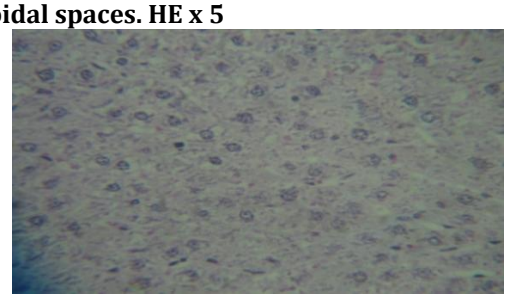
(Intoxicated control 24gm/kg Ethanol)

Figure 3. Higher magnification of control rat liver section revealing swollen hepatocytes with decreased sinusoidal spaces. HE x 5



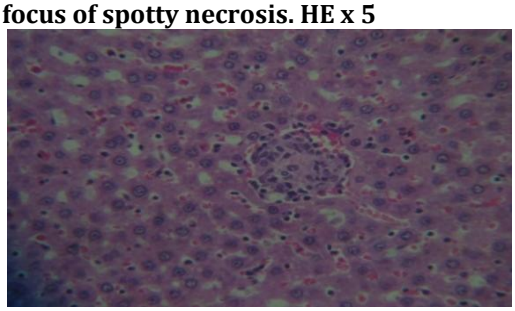
(Ethanol 24gm/kg + A.paniculata 50mg/kg)

Figure 4. Higher magnification of control rat liver section revealing swollen hepatocytes with decreased sinusoidal spaces. HE x 5



(Ethanol 24gm/kg + A.paniculata 100mg/kg)

Figure 5. High dose rat liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis. HE x 5



(Ethanol 24gm/kg + A.paniculata 200mg/kg)

DISCUSSION

Hepatoprotective activity of herbal formulation containing different proportion of *Andrographis paniculata* was evaluated using hepatotoxicity model. Ethanol induced toxicity model was taken as prototype of exudative. The extracts of *Andrographis paniculata* successive water extracts showed significant hepato protective effect. From the above experimental study we conclude that: The extract of *Andrograrphis paniculata* produces adequate hepatoprotective activity on albino wistar rats. It showed potent hepatoprotective activity.

It has been found that aqueous extract of *Andrographis paniculata* could prevent ethanol-induced biochemical changes of liver toxicity. The hepatoprotective activity of the AEAP was monitored by estimating serum AST, ALT, LDH, ALP, Tot. Bilirubin, Dir. Bilirubin, which give a good idea about the functional state of the liver. The increase in the levels of serum bilirubin reflected the level of jaundice and ALP was the clear indications of cellular leakage and loss of functional integrity of cell membrane.

Ethanol induced hepatotoxicity when administered into the body. In the present study, the evaluation has been carried out for extract of *Andrographis paniculata.* Our experiment showed the extract of *andrographis paniculata* successive water extracts posses the good hepatoprotective activity.

The dose levels selected for studies were *A. paniculata* extract of 50 mg/kg, 100mg/kg and 200mg/kg for successive water extract. This ability of AEAP to protect the liver from ethanol-induced damage might to be attributed to this ability to restore the activity of anti-oxidative enzymes.

CONCLUSION

Hepatoprotective activity of the aqueous extract of *Andrographis Paniculata* was studied. In this study, The extract of *Andrograrphis Paniculata* produced adequate hepatoprotective activity on albino wistar rats. The dose levels selected for studies were 50 mg/kg, 100mg/kg and 200mg/kg. Histopathological examination of the liver section of the rats treated with toxicant showed intense centrilobular necrosis and vacuolization. The rats treated with extracts along with toxicant showed sign of protection against these toxicants to considerable extent as evident from absence of necrosis and vacuoles. Thus it was concluded that the extract exhibited significant dose dependent hepatoprotective activity.

REFERENCES

1. Essabu N A, Bajit M L, Farhood A, Vonderfeeht S L and Jaschke H; Transcriptional activation of vascular cell adhesion molecul 1 geneion vivo and its role in the pathophysiollgy of neutrophil induced liver injury in murine endotoxin shcok. J. Immaunol. 1997; 1458, 5941-5948.
2. Kestersib J W, Graneman G R, and Machinist J M; The hepatotoxicity of valproic acid and its metabolities in rats. Todiciological biochemical and histopathologic studies, Hepatology. 1984; 4, 1143-1152.
3. Jaeschke H, Smith C W, Clemens M G, Ganey P E and Roth R A; Mechanisms of inflammatory liver injury: Adhesion molecules and cytotoxicity of neutrophils. *Toxicol. APPL. Pharmacol.* 1996; 139, 213-226
4. Chen J H Jiang; Morphological and histological studies on Andrographis Paniculata(Burm.F.) Nees. And comparison with four other Acanthaceae herbs.Yao Xue Xue Bao 1980 Dec;15(12):750-760.
5. Hu C Q, Zhou B N, Isolation and structure of two new diterpenoid glucosides from Andrographis Paniculata Nees. Yao Xye Xue Bao 1982 Jun;17(6):435-440.
6. Rana A C, Avadhoot Y; Hepatoprotective effects of Andrographis Paniculata against carbon tetrachloride-induced liver damage.College of pharmacy,Shri Govindram Sekseria Institute of Technology and Science,Indore,India.Arch Pharm Res 1991 Mar;14(1):93-95.
7. Wang H W, Zhao H Y, Xiang S Q; Effects of Andrographis Paniculata component on nitric oxide,endothelin and lipid peroxidation in experimental atherosclerotic rabbits,Zhongguo Zhong Xi Yi Jie He Za Zhi 1997 Sep;17(9):547-549.
8. Hancke J L, Burgos R A, Wikman G K; Prevention of common colds with Andrographis Paniculata dried extract. A pilot double blind trial.Phytomedicine. 1997; 4(2):101-104.
9. Sandberg F; Andrographidis herba Chuanxinlian: A review Gothenburg,Sweden:Swedish Herbal Institute. Available from the American Botanical Council 1994.
10. Morimoto M, Zern M A, Hegbjork A L ingelman, Sundberg M and French S W; Fish oil, alcholol, and liver pathology.Role of cytochrome P450 2E1 Proc. Soci. Exp. Biol. Med. 1994; 207, 197-205.
11. Essani N A, Fisher M A, Fathood A, Manning A M, Smith C W and Pcssayre D; Cytokinc induced hepatic intercellular adhesion molcule, 1 (ICAM-1) meassenger RNA expression and its role in the pathophysiology of murine endotoxn shock and acute liver failure, Hepatology. 1995; 21, 1632-1639.
12. Jaeschke H, and Smith C W; Mechanisms of neutrophil-induced parenchymal cell injury. *J.Leukoc. Biol.* 1997; 61, 647-653.
13. Bergmeyer H U, Bowers G N Jr, Hørder M, and Moss D W; Provisional recommendations on IFCC methods for

the measurement of catalytic concentrations of enzymes. Clinical Chemistry 1977; 23, 887-899.

14. Laskin D L and Laskin J D; Role of Macrophages and inflamatory mediators in chemically induced toxicity, Toxicology. 2001; 160,111-118.
15. Young D; In Effect of Pcanalytical Variables on clinical Laboratory Tests, 2nd ed., AACE Press, Washington, 1997, 4-489.
16. Mess D W, Henderson A K; Clinical Enzymdogy in Tietz Textbook of clinical chemistry, 3rd ed. C A Buritis and E R Ashwood, Eds W B Saunders, Philadephia, 1994, 617-721.
17. Murray R L; Enzymes in clinial chemistry; Theroy, analysis and co-relation, L A KaPlan and A J Pesece, Eds. C V Mesby, Toronto, 1994, 1079-1134.
18. Enzymes, Kaplan A, Lavemel L S, In clincal Chemistry; Interpretation and Techniques, 2nd ed., lea and Febiger, Ph8iladelphia, 1983, P219-296.
19. Friedman S L; Molecular regulation of hepstic fibrosis, an integrated cellular response to tissue injury J lBiol Chem, 2000; 275, 2247-2250.