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Potential action of *Andrographis paniculata* against Chronic Ethanol Consumption Induced Liver Toxicity in Experimental Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Authors SV and AM designed the experimental protocol, carried out experimentation and drafted the manuscript. Author SV performed the statistical analysis and interpreted the results. Both authors read and approved the final manuscript.

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ABSTRACT

Objective: To determine the ameliorative potential of methanolic extract of *Andrographis paniculata* to ethanol induced wistar rats, and its possible mechanism of action. Chronic ethanol consumption is a major risk factor in determining liver disease and modulates the risk factors for metabolic syndrome via multiple mechanisms, including the regulation of the lipid metabolism.
Methods: Male wistar rats were divided into 6 groups (n=6/group), and fed with a standard diet, ethanol, and ethanol supplemented with extracts for 6 weeks. The ethanol changes were determined via a serum enzyme profile and damage in tissues of the liver. Liver toxicity was assessed by means of serum lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, and a liver histopathological investigation.
Results: Oral administration of 100 mg/kg and 200 mg/kg of methanolic extract of *A. paniculata* (MEAP) offered a significant (P=0.05) dose dependent protection against ethanol induced hepatotoxicity.
Conclusions: The present study revealed that MEAP in the phytochemical constituents plays an active role against enzymes elevation and liver protection.

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Keywords: Andrographis paniculata; ethanol; hepatotoxicity; serum enzymes; liver protection.

1. INTRODUCTION

Chronic alcohol consumption is a known risk factor for liver disease, which represents a major cause of morbidity and mortality worldwide. It is involved with almost all biochemical pathways [1]. Liver injury caused by toxic chemicals and certain drugs have been recognized as a toxicological problem. Animal models suggest that liver injury in chronic alcoholics is due to oxidative stress that leads to fibrosis, impaired liver functions and increased apoptosis [2]. Research has linked chronic alcohol consumption and a variety of pathological conditions ranging from simple intoxication to severe life threatening pathological states [3]. The pathological process of alcohol-induced liver disease is characterized by morphological changes with minimal injury to more advanced liver damage, including steatosis, fibrosis and cirrhosis [4,5]. Herbs play a role in the traditional treatment and management of various liver disorders [6,7]. *Andrographis paniculata* is used extensively in the Indian traditional (Ayurvedic) system of medicine [8]. With this species it is mostly the leaves and roots that have traditionally been used in Asia and Europe as a remedy for a wide spectrum of ailments or as an herbal supplement for health promotion [9]. The Indian pharmacopoeia narrates that it is a prominent constituent in more than 26 Ayurvedic formulations [10]. *Andrographis paniculata* was selected by the Ministry of Health as one of the medicinal plants to be included in "The National Drug List of Essential Drugs in 1999" (list of herbal medicinal products) in Thailand [11]. The taste of its leaves is very bitter [12], and relates to various pharmacological properties such as antiviral [13], anti-inflammatory [14], antivenom [15], immunostimulatory [16], anticancer [17], anti-HIV [18], anti-allergic [19] and hypoglycemic activity [20]. It grows abundantly in southeastern Asia, i.e., Sri Lanka, Pakistan, Malaysia and Indonesia, and extensively in India, China and Thailand [21]. Previous Investigation on the chemical composition of *A. paniculata* showed that it is a rich source of diterpenoids and 2'-oxygenated flavonoids, including andrographolid [22], Neoandrographolide [23], 14 deoxy-11,12 didehydroandrogra pholide [24], 14-deoxyandro-grapholide [25], Isoandrographolide [26], β-D-glucoside [27], Homoandrographolide [20], Andrographan [28], Andrographosterin [29] and stigma-sterol [30]. Silymarin administration has demonstrated normalization of serum liver enzyme and total bilirubin levels in patients with alcoholic liver disease; there was also improvement in liver tissue histology [31]. Administration of ethanol produces a decrease in the hepatic content of glutathione (GSH), which is an important biomolecule that affords protection against chemically-induced cytotoxicity [32]. This study investigated the protective effect of combination of ethanol with 100 and 200 mg/kg of MEAP influence on regulating the normal metabolic function and protection of hepatic damage in rats, to provide a definitive theoretical and experimental basis for its clinical application.

2. METHODS

2.1 Collection and Preparation of the Plant Materials

The leaves of *A. paniculata* were collected in April and May from southern hill stations in Thirunelveli District, Tamil Nadu, India. Botanical identification was carried out at the Department of Pharmacognosy, OPJS University, India. A voucher specimen No. OPJS/2013/11 has been deposited in the museum of the Department of Pharmaceutical Sciences, OPJS University, Churu. The leaves were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40 mesh sieve. The powdered plant materials were stored in an airtight amphour glass bottle.

2.2 Drugs and Chemicals

Enzymes kit was obtained from Span Diagnostics Ltd. Surat, India. Silymarin was purchased from Sigma Aldrich Chemical China. All other chemicals were of analytical grade procured from reputed Indian manufacturers.

2.3 Experimental Animals

The experimental design was approved by the institutional animal ethical committee of OPJS University, Churu, Rajasthan, India. Male Wistar rats 8-10 weeks old, weighing 180-215 g, and kept under standard conditions temperature (25±5℃), relative humidity (55±1 − %), 12 hrs light and dark cycles, and fed with standard pellet diet and water *ad libitum*.

2.4 Preparation of the Extracts

The dried powder was extracted sequentially by hot continuous percolation method using soxhlet apparatus for 24 hrs. The solvent from the extracts was recovered under reduced pressure using a rotary evaporator and subjected to freeze drying in a lyophilizer untill a dry powder was obtained.

2.5 Acute Toxicity Study

Acute oral toxicity study was performed as per Organization of Economic Cooperation and Development (OECD) guideline 423. Each group consists of 8 rats. Different doses of fed to the rats by oral intubation. The animals were observed individually every 30 minutes after dosing for the first 24 hrs thereafter daily for 14 days. The time at which signs of toxicity appear/disappear was observed systematically and recorded for each animal.

2.6 Experimental Design

A total of thirty six rats were equally divided into 6 groups of six each. Group I served control without any treatment. Group II served as an ethanol control. Animals of groups III, IV, V and VI were administered ethanol followed by Silymarin and different doses (50,100 and 200 mg/kg) of *A. paniculata* respectively for 6 weeks.

Group I: Control (10 ml/kg normal saline, bw po.)
Group II: Ethanol (20%v/v/kg bw po.)
Group III: Ethanol + Standard drug of Silymarin (50 mg/kg bw po.)
Group IV: Ethanol + Methanolic extract of *A. paniculata* (50 mg/kg bw, po.)
Group V: Ethanol + Methanolic extract of *A. paniculata* (100 mg/kg bw, po.)
Group VI: Ethanol + Methanolic extract of *A. paniculata* (200 mg/kg bw, po.)

2.7 Histopathological Study

At the end of the study, all rats were euthanasia by cervical dislocation after overnight fasting. Liver tissue was separated for further investigation. Liver slices were fixed in 10% formalin and embedded in paraffin wax. Sections of 5 µm thickness were made using a microtome and stained with haematoxylin- eosin. The rats were observed under a light microscope and photographs of each slide were taken at 40× magnification.

2.8 Statistical Analysis

The data are presented as mean ± standard error mean (SEM) for six animals in each group. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA), followed by Duncan test. Significant differences were set at *P* values lower than 0.05. Values that have a different superscript letter (a, b, c, d, e) differ significantly with each other (P=0.05).

3. RESULTS

3.1 Acute Toxicity Study

After administration of 2000 mg/kg dose of methanolic extract of *A. paniculata*, the animals showed no behavioral abnormality, dyslipidemia, toxic effects or mortality. Hence, *A. paniculata* at doses of 50, 100 and 200 mg/kg, p.o. was selected for further pharmacological investigations.

Table 1. Effect of MEAP on serum enzymes profile in control and experimental rats

Groups	LDH (IU/L)	ALP (IU/L)	Total Bilirubin (IU/L)	Direct Bilirubin (IU/L)
Normal control	1168.325±12.019^{a}	171.562±1.290^{a}	0.117±0.004^{a}	0.095±0.007^{a}
Toxicity control	2400.778±322.097^{b}	337.942±9.333^{b}	0.452±0.038^{b}	0.280±0.002^{b}
Standard drug	1168.893±18.288^{a}	192.740±10.496^{c}	0.127±0.006^{a,c}	0.114±0.006^{c}
Low dose	1944.398±111.281^{c}	296.440±12.556^{d}	0.423±0.036^{d}	0.252±0.006^{d}
Medium dose	1668.077±117.888^{d}	232.285±19.048^{e}	0.208±0.021^{e}	0.165±0.005^{e}
Higher dose	1253.113±91.790^{a}	188.645±8.693^{c}	0.148±0.008^{c}	0.127±0.003^{f}

The data are presented as mean ± standard error mean (SEM) for six animals in each group. Statistical analysis of the data was performed using one-way analysis of variance (ANOVA) followed by Duncan test. Significant differences were set at P values lower than 0.05. Values that have a different superscript letter (a, b, c, d, e) differ significantly with each other (P= 0.05).
Abbreviation: LDH: lactate dehydrogenase; ALP: alkaline phosphatase

3.2 Pharmacological Interventions on Liver Enzymes Profile

The ethanol treatment for 6 weeks caused a significant increase in serum LDH, ALP, Tot. Bilirubin, Dir.Bilirubin levels when, compared to rats fed with a standard diet. After treatment with MEAP 100 and 200 mg/kg and Silymarin, 50 mg/kg shows a significant *(P=0.05)* response against ethanol induced hepatic damage.

3.3 Pharmacological Interventions on Histology of the Liver

Ethanol treated rats change in hepatic tissue architecture such as micro and macro-vascular steatosis, increased fatty infiltration, inflammation (over activation of kupffer cells), sinusoidal dilation, degeneration of central vein and vacuolization. Treatment with MEAP 200 mg/kg effectively attenuated these effects (Fig. 1).

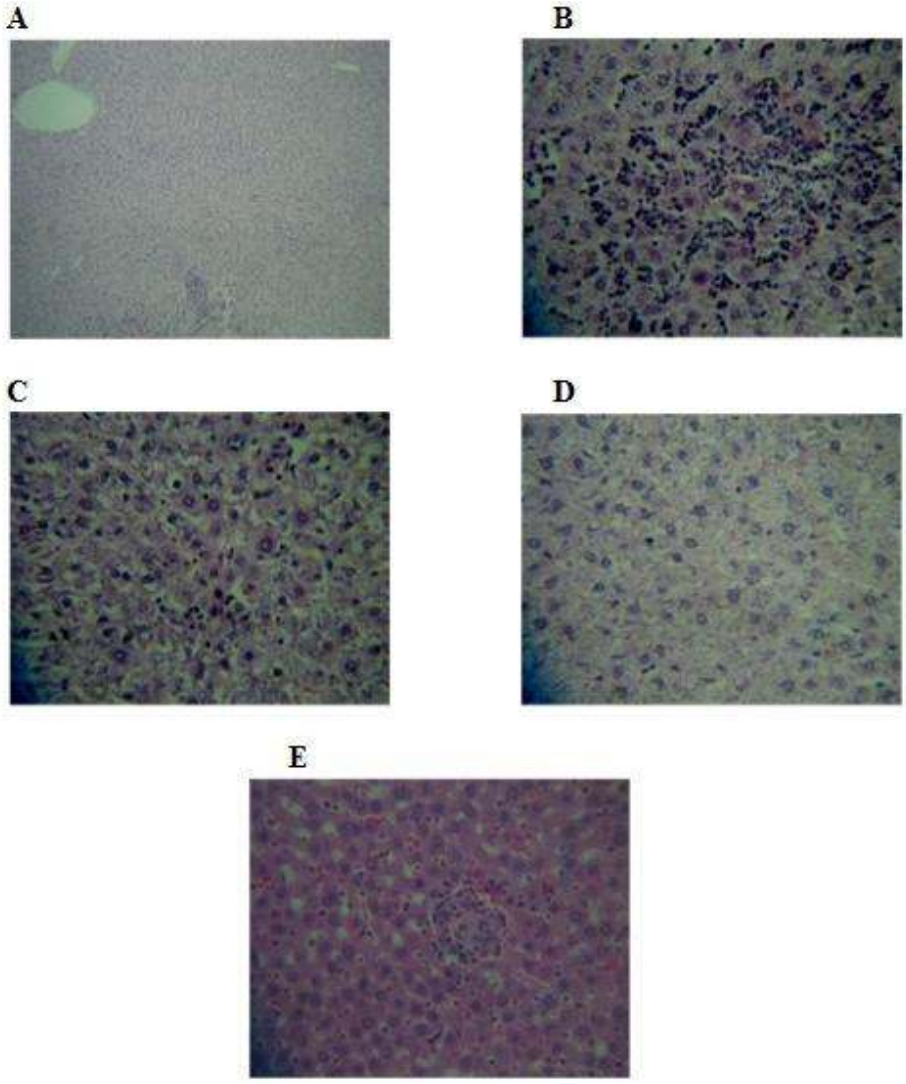

Fig. 1. Effect of various pharmacological interventions on histology of liver tissue

A: Control rat liver section revealing normal hepatic parenchyma with a central vein at the top corner; B: A focus of intense necrotic hepatitis in the diseased control revealing nuclear pyknosis, karyolysis/karyorhexis and intense cellular infiltration; C: Standard drug treated liver section revealing relatively normal hepatic parenchyma comparable to that of the control; D: Higher magnification of control rat liver section revealing swollen hepatocytes with decreased sinusoidal spaces; E: High dose rat liver section revealing comparatively normal hepatic parenchyma with a single focus of spotty necrosis.

4. DISCUSSION

Previous reports demonstrated that alkaloids [33], tannins [34], flavonoids [34], amino acids [35], glycosides [36] and terpenoids [12] may protect against hepatic damage. Our results indicated that the phytochemical constituents of MEAP play an important role of hepatoprotective activity. Acute toxicity studies of *A. paniculata* up to 2000 mg/kg were found to be non-toxic and did not cause any death of the tested animals. Previous data indicated that polyphenolic compounds may protect against oxidative damage and have anti-inflammatory activity [14]. Several epidemiological studies have shown flavonoid intake with a low risk of liver toxicity [37,38]. A previous study reflected that flavonoids and glycosides compounds may protect against oxidative damage [39]. Most recent researches have focused on the health aspects of flavonoids for humans. Previous studies shown that flavonoids to have antioxidative activity, free radical scavenging capacity, coronary heart disease prevention, hepatoprotective, anti-inflammatory, and anticancer activities, while some flavonoids exhibit potential antiviral activities [40,41]. In plant systems, flavonoids help in combating oxidative stress and act as growth regulators [42,43].

Table 1 shows the LDH in ethanol treated rats a significant rise in serum activities comparable to non−treated rats (control). An increase in the liver enzymes may attribute to the damaged structural integrity of the liver, which results in the leakage of these enzymes from the cytosol into the bloodstream [44]. The present study proved that different doses of MEAP markedly decreased lactate dehydrogenase level (P=0.05) when, compared to ethanol treated rats. Sturgill and Lambert [45] reported that long-term alcohol consumption does not only activate free radical generation, but also alters the levels of both enzymatic and non-enzymatic endogenous antioxidant systems. This results in oxidative stress with a cascade of effects leads to affecting both functional and structural integrity of cell and organelle membranes [46]. Our investigation shows ethanol treated rats had significantly associated with cellular necrosis and an increase in serum levels of many biochemical markers like ALP and bilirubin. Wolf found that the oxidative stress is highly correlated with a wide variety of inflammatory and metabolic diseases [47]. Moreover, Feroz and Nahida showed that phytochemical constituents of glycosides, terpenoids, flavonoids protect the membrane from damage through the oxidative damage of ethanol [48]. Consistently, we found that MEAP decreased ALP, LDH and Bilirubin levels. The study of Lodhi et al. demonstrated that elevation of these biochemical enzymes caused the damage in hepatocytes. The present study revealed MEAP on reduction of elevated enzyme and protection of liver damage in rats [49].

Chronic administration of ethanol also causes microsomal enzyme induction in animals [50]. We found ethanol treated rats decreasing in the level of serum LDH and ALP which is in line with an earlier study [51]. Chronic consumption of ethanol is crucial for the development of total and direct bilirubin levels. Passive hepatic congestion due to increased central venous pressure may cause elevations of liver enzymes and both direct and indirect serum bilirubin. This condition may be an initiate the etiology of hepatic damage [52,53]. In the present study, MEHI on the ethanol treated rats indicate the lowering effect serum enzymes may be due to the inhibition of hepatic cholesterogenesis [54,55]. Moreover, the rats received a MEAP at the dose of 100 and 200 mg/kg exhibited a reduction in LDH, ALP and Bilirubin levels. Trivedi et al. [56] showed chemical toxicated rats had a significant increase in the level of liver enzymes. Kim et al. [57] reported hepatic steatosis is a common consequence of obesity, and its prevalence has been further characterized with hepatic fat accumulation. Ethanol can also sensitize cells, causing cell populations downstream of inflammatory cytokine signaling to respond more robustly [58]. Our findings demonstrated that MEAP reversed the effect of ethanol induced liver damage.

5. CONCLUSION

Our study showed that chronic intake of ethanol ameliorated hepatic damage in experimental rats. We found that among the dose group of methanolic extract of *Andrographis paniculata* (MEAP) 100 and 200 mg/kg had a potential effect on inhibiting the progression of hepatotoxicity in ethanol-fed rats. The results reflected may have beneficial and reducing risk factors for liver disease.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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