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HEPATOPROTECTIVE ACTIVITY AND ANTI-OXIDANT ACTIVITY OF ANTHOCEPHALUS INDICUS IN EHTANOLINDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

The effect of *Anthocephalus indicus* (*A.*indicus) was studied on ethanol induced (3ml/kg body wt.) liver damage in rats. A marked increase in serum levels of Aspartate transaminase (AST), AlkalinePhosphatase (ALP), Glutamate pyruvate transaminaseactivity (GPT) and total bilirubin, accompanied with reduction of superoxide dismutase, catalase levels in liver was observed. The alterations in liver enzymes wereassociated with the formation of lipid peroxides in liver tissue. Treatment with powdered flowers of *Anthocephalus indicus*(500mg/kg)was found to protect the rat from hepatotoxic action of ethanol as evidenced by significant decrease in serum levels of AST, ALP, GPT and total bilirubinas well as significant increase in hepatic superoxide dismutase and catalase activities and significant reduction in lipid peroxides. Hepatic enzymes levels as well as anti-oxidant enzyme levels were recovered partially on treatment with *A.indicus.*

Keywords: *Anthocephalus indicus*, Ethanol, Hepatoprotective activity, Antioxidant activity.

INTRODUCTION

Alcoholic liver disease is a worldwidehealth problem. The recognized forms of alcoholic liver diseases are fatty liver/steatosis, alcoholic hepatitis and liver cirrhosis (Tariq Mahmood *et al*., 2009). Various studies described that ethanol caused accumulation of reactive oxygen species like super oxide, hydroxyl radical (Younes M *et al*., 1987) and hydrogen peroxide in hepatocytes that oxidized the reduced glutathione, which in turn lead to lipid peroxidation of cellular membrane, and oxidation of protein and DNA resulting in hepatic injury (Cederbaum Al *et al*., 1989). Ethanol-induced hepatotoxicity has been reported to cause significant increase in the serum values of ALT, GPT, AST and total bilirubin (Tariq Mahmood *et al*., 2009).

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In the absence of reliable liver protective drugs in allopathic medicine, herbs play a role in the management of various liver disorders. Anumber of plants have shown hepatoprotective property. *Anthocephalus indicus* (Family: Rubiaceae) known as Kadamand its roots, bark, leaves, flowers and fruits have beenused in Aryuvedic remedy for the treatment of fever, anemia, uterine complaints, menorrhagia, blood and skin diseases, diarrhea, stomatitis and dysentery.*A.indicus* has been reported to possess antimicrobial (Umachigi *etal*., 2007), wound healing (Umachigi *et al*., 2007), antioxidant (Kumar V *et al*., 2010), hypolipidemic (Vishnu Kumar *et al*., 2010), ant diabetic activity (Kumar *et al*., 2008) and hepatoprotective activity (Kapil A *et al*., 2006). There are reports that heartwood, leaves, flowers, and seeds of *A.indicus* contain typical alkaloid: Cadambine and its derivatives, some complex polysaccharides and other constituents (Shaua NP *et al*., 2000). Chlorogenic acid which was isolated from *A.indicus* possesses hepatoprotectiveactivity (Kapil A *et al*., 2006)

which causes reversal in lipid peroxidation, enhancement of cellular antioxidant defense system.

Herbal formulations are preferred due to lesser side effects and their low cost. One of the etiological factors implicated in the development of liver disorders is by free radicals. Thus, a drug having multi-fold properties such as hepatoprotective and antioxidant activities is in great demand. Therefore in the present study, an attempt has been made to explore hepatoprotective and antioxidant activitiesof *Anthocephalus indicus.*

MATERIALS AND METHODS

Ethanol was obtained from local chemical store and standard drug Silymarin was obtained from Sigma Aldrich Company.

Preparation of drug material

The powdered flowers of *A.indicus*were prepared as suspension in 10% Sodium carboxy methyl cellulose. Chlorogenic acid is the main constituent and the antioxidant property is the active principle of the plant.

Animals

Male wistar rats (180-200g)) were selected; they were housed under controlled environmental conditions room temperature 25 ± 1^{o}C with 12hr light and dark cycle, treated with standard pellets and tap water.

Study design

Animals were divided into fourgroups each containing six rats allotted to different treatment groups, Group 1 served as control group. Group 2 rats receivingethanol (60% v/v ethanol, 30ml/kg body wt.) oral route for 30 days. Group 3 rats receiving ethanol and Silymarin (200mg/kg body wt) (Tariq Mahmood *et al*., 2009) standard drug give via oral route for 30 days. Group 4 rats receiving ethanol and *A.indicus (*500mg/kg body wt) (Kumar V *et al*., 2008) oral route for 30days.

Biochemical analysis

On the 30th day animals were anesthetized 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, GPT levels.

Liver homogenized (10% w/v) in cold 1M phosphate buffer (p^{H}7.2) used for estimation of melanaldehyde (MDA) levels, 0.15 M KCl was also used for the estimation of superoxide dimutase(SOD) and catalase (CAT).

Statistical Analysis

One way analysis of variance (ANOVA – Student's t test) was performed by the comparison of values for ethanol treated group with control, ethanol and drug treated with ethanol only. P<0.05 was considered statistically significant and the results were expressed as mean±SEM

RESULTS

Effect of *A.indicus* and Silymarin on serum ALT, AST, ALP, GPT levels in ethanol induced hepatotoxicity in rats

Table 1. Table showing the administration of ethanol markedly increased the serum AST, ALP, GPT and total bilirubin and the treatment with *A.indicus* and Silymarin reduced the serum levels AST, ALP, GPT and total bilirubin

Groups	AST (IU/mL)	ALP (IU/mL)	GPT (IU/mL)	Total bilirubin (µmol/L)
Control	9.5 ± 10.1	103.3 ± 3.3	21.4 ± 5.3	1.2 ± 0.13
Ethanol treated	30.6 ± 8.3***	200.7 ± 4.3**	40.6 ± 3.6***	3.0 ± 0.24**
Ethanol+ Silymarin	11.6 ± 7.5**	125.8 ± 9.8**	29.5 ± 6.6**	1.7 ± 0.15**
Ethanol+ *A. indicus*	15.7 ± 6.6**	140.7 ± 10.3*	32.0 ± 8.3**	1.9 ± 0.11*

Values are expressed as mean±SEM; n=6

Ethanol treated group was compared with control, Ethanol and drug treated with ethanol. $^{***}P$ <0.001, $^{**}P$<0.01,$^{*}P$<0.05

Effect of *A.indicus* and Silymarinon hepatic SOD, Catalase and MDA in ethanol induced hepatotoxicity in rats

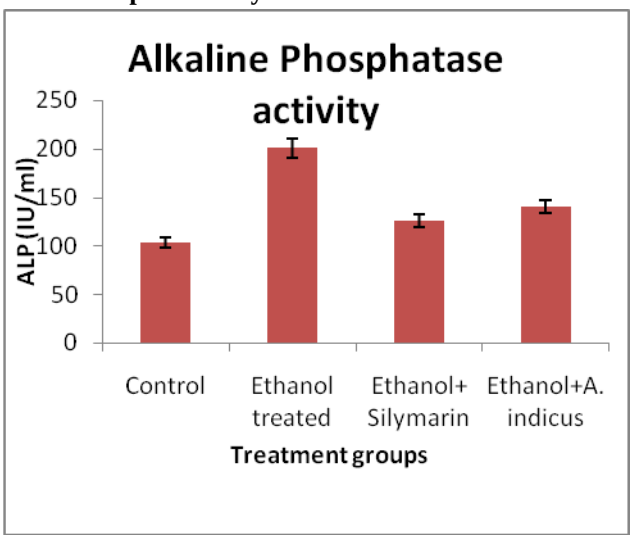
Table 2. Table showing the administration of ethanol has decreased the liver SOD, Catalase levels and significantly increased the liver MDA levels. Treatment with *A.indicus* and Silymarin has shown improvement in the enzymes levels

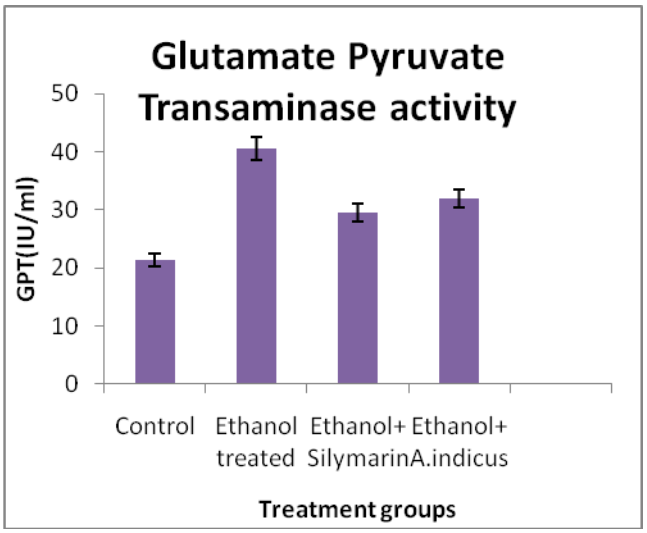
Groups	SOD (Unit/min/mg protein)	Catalase(Unit/min/mg protein)	MDA (µg/mL)
Control	3.9 ± 0.20	387 ± 101.8	50.5 ± 10.7
Ethanol treated	1.7 ± 0.30**	190 ± 90.7***	92.1 ± 12.5**
Ethanol+Silymarin	2.6 ± 1.50**	290 ± 88.3**	68.3 ± 9.8**
Ethanol+ *A.indicus*	2.3 ± 1.70**	279 ± 99.7**	73.6 ± 13.3*

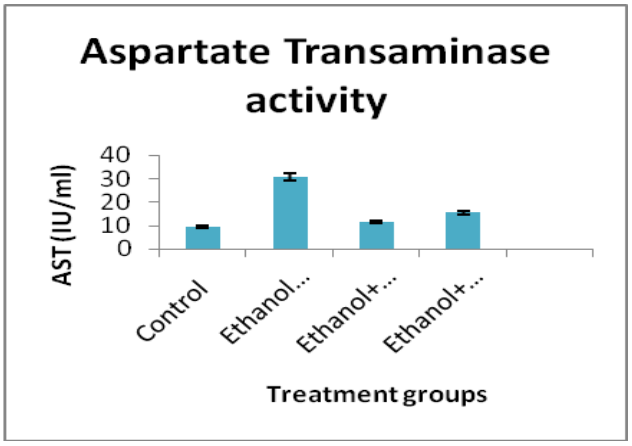
Values are expressed as mean±SEM; n=6

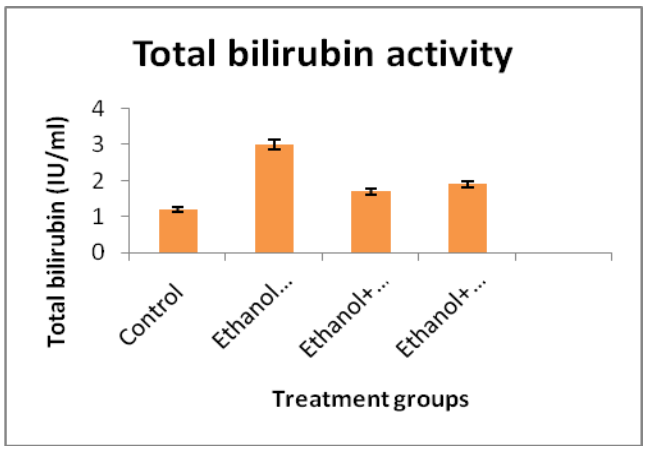
Ethanol treated group was compared with control, Ethanol and drug treated with ethanol. $^{***}P$ <0.001,$^{**}P$<0.01,$^{*}P$<0.05

Graph 1. Showing the Effect of *A.indicus* and Silymarin on serum ALP, GPT, AST, Total bilirubin levels in ethanol induced hepatotoxicity in rats

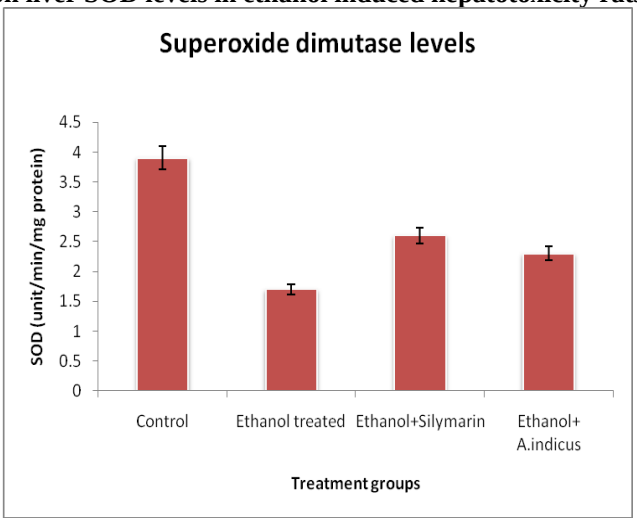




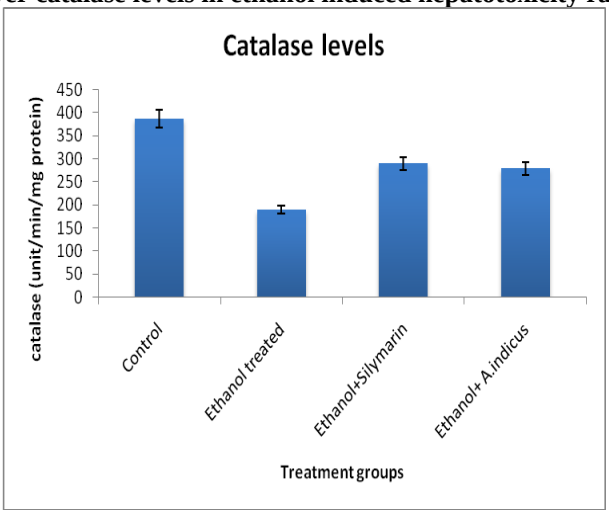




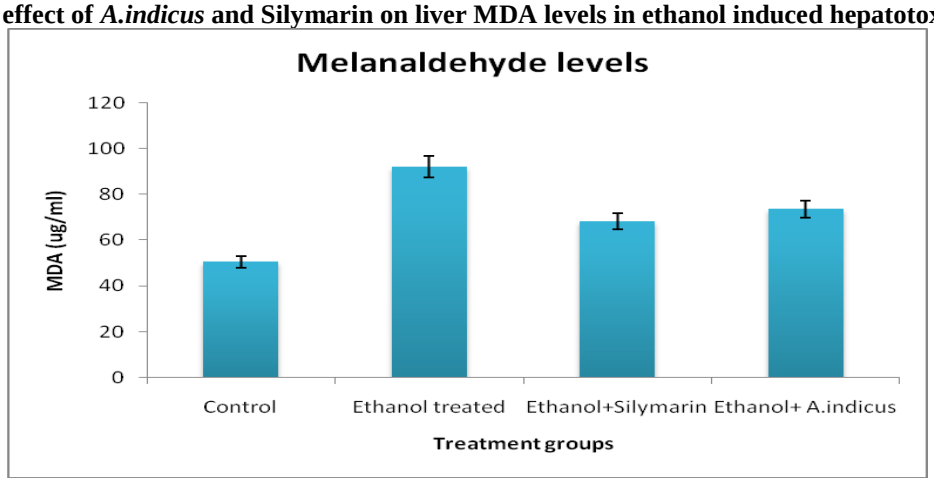
Graph 2. Showing the effect of *A.indicus* and Silymarin on liver SOD levels in ethanol induced hepatotoxicity rats



Graph showing the effect of *A.indicus* and Silymarin on liver catalase levels in ethanol induced hepatotoxicity rats



Graph 3. Showing the effect of *A.indicus* and Silymarin on liver MDA levels in ethanol induced hepatotoxicity rats



DISCUSSION

The serum levels of AST, GPT, ALP and total bilirubin in ethanol induced animals was significantly increased as compared to those animals in the control group. This may be due to production of reactive oxygen species (ROS), inducing protein oxidation and lipid peroxidation which resulted in hepatocyte injury (Enomoto 2003), causes the leakage of enzymes from hepatic cells due to altered permeability results in increased levels of AST, GPT, ALP and total bilirubin in serum.

In 2006 Kapil *et al*., studied the hepatoprotective activity of chlorogenic acid isolated from *Anthocephalus indicus*. Chlorogenic acid caused a significant reversal in lipid peroxidation, enzyme leakage and is responsible for its liver protective activity causing decreased leakage of enzymes from hepatic cells resulted in decreased levels of enzymes in serum. *A.indicus* exhibited a therapeutic protective action in ethanol administered rats.

The tissue SOD, Catalase levels in ethanol induced rats was significantly decreased as compared to control animals. This may be due to production of ROS which induce lipid peroxidation which was evidenced by raised MDA levels in ethanol treated rats. In 2006 Kapil *et al*., in 2010, Vishnu *et al*., studied the antioxidant property of *A.indicus.* Due to its antioxidant property resulted in increased levels of tissue SOD, Catalase in liver. Chlorogrenic acid which reduced the lipid peroxidation in liver resulted in decreased levels of tissue MDA levels in ethanol treated rats.

CONCLUSION

The results in the present study demonstrated the hepatoprotective activity and anti-oxidant activity of *A.indicus.* The hepatoprotective activity and anti-oxidantmight be due to inhibition of tissue lipid peroxidation and activation of tissue SOD and Catalase. The beneficial effects might be due to chlorogenic acid and its antioxidant property. Moreover, further studies on drug metabolism, in vivoand in vitro of *A.indicus*of various fractions are under progress to sustain the present study.

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