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Hepatoprotective activity of *Saccharum officianarum* against ethyl alcohol induced hepatotoxicity in rats

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Abstract

Liver is a highly sensitive organ. Liver damage was induced in wistar rats by administration of 20% Ethanol for eighteen days and *Saccharum officinarum* was given for eight to eighteen days. Silymarin was used as reference drug. Levels of different marker enzymes were estimated in serum. A probe into the mechanism of action was attempted by estimating total protein, malondialdehyde and glutathione levels in liver homogenates in order to evaluate the degree of lipid peroxidation. Histopathological studies were also done to confirm the biochemical changes. *In vitro*, hepatocytes of control group were exposed to ethanol, while cell suspensions were pretreated with *S. officianrum* (1mg/ml) before exposure to ethanol and standard group received silymarin (1mg/ml) before exposure to ethanol. While *in vitro* model, estimation of percentage viability of hepatocytes was evaluated by trypan blue assay. Ethanol produced significant changes in physical (increased liver weight and volume), biochemical (increase in serum alanine transaminase, aspartate transaminase, alkaline phosphatase, direct bilirubin, total bilirubin and triglycerides), liver tissue (decreased total protein, GHS and increased MDA), histological (hepatocytes damage) and functional (thiopentone induced sleeping time) liver parameters. Treatment with *S. officinarum* juice significantly prevented the physical, biochemical, histological and functional changes induced by ethanol in the liver, while *in vitro* model exhibited significant increase in % viability of cells with pre exposure to *S.officinarum* and Silymarin as compared to ethanol control. Juice of *S. officinarum* exhibited significant hepatoprotective action against ethanol induced hepatic injury both in vivo and in vitro study.

Keywords: Ethanol, hepatoprotective activity, juice, *Saccharum officinarum*, antioxidant.

Introduction

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction. [1] The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. In spite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [2, 3]. Management of liver disease is still a challenge to the modern medicine. Presently only a few hepatoprotective drugs and that too from natural sources (there is not a single effective allopathic medication), are available for the treatment of liver disorders.
The *Saccharum officinarum*, family Poaceae, has been indicated as cardiotonic, expectorant, haemostatic, tonic, diuretic and useful in urinary disorders in shadangdhar samhita and bhavprakash. The present study was undertaken to evaluate the hepatoprotective activity of these plants in experimental animal. The plant contains amongst many others flavonoids, alkaloids, amino acids, carbohydrates, starch and vitamins. The plant has better antioxidant effects. [4]

Materials and Methods

Drugs and chemicals

Kits for all biochemical estimations were purchased from Transasia Biomedicals Ltd., Daman, India. Silymarin (Micro Labs, Bangalore)

Animals

Wistar strain albino rats (150-200 g) of either sex maintained under standard husbandry conditions were used for all sets of experiments comprising of six rats each. The rats were allowed of take standard laboratory feed and water *ad libitum.* The experimental protocol was approved by Institutional Animal Ethical Committee as per the guidance of committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Approval No. project 6006 dated 20th Nov 2006).

Plant collection

Saccharum officinarum was obtained from a commercial supplier of Ahmedabad and it were identified and authenticated by Dr. G. C. Jadeja, Professor and Head, Department of Agricultural Botany, B. A. College Of Agriculture, Anand Agriculturural University, Anand, Guajrat, India.

Preparation of plant juice

Cane of *S. officinarum* was milled between two rollers and juice thus obtained was strained through a muslin cloth.

Hepatoprotective studies

A total of 24 animals were equally divided into 4 groups (6 nos. in each group). Group 1 which served as Normal control received only vehicle for 18 days. (Distilled water, p.o) whereas Group 2,3 and 4: received 20% Ethanol (3.76 gm/kg/day, p.o.) for 18 days. In addition group 3 and 4 received reference standard Silymarin (100mg/kg., p.o.) and *Saccharum officinarum* juice

(0.75ml/100gm., p.o.) respectively for 8th days to 18th days. On day eighteenth, thiopentone sodium (40 mg/kg, i.p) was injected and the sleeping time was recorded for all the animals. At the end of the treatment, blood samples were collected by retro orbital puncture and the serum was used for the assay of marker enzymes *i.e.* serum glutamic oxalacetic transaminases (SGOT), serum glutamic pyruvic transaminase [8] (SGPT), serum alkaline phosphatase [9](ALP), serum total bilirubin [10] (STBiI), serum direct bilirubin [10] (SDBiI) and triglyceride which reflected the functional state of the liver analyzed according to the reported methods.

The rats were sacrificed by cervical dislocation, immediately after the collection of blood, the animals were euthanized with an over dosage of ether; their livers were removed, washed in saline and the wet weight and volume was determined. The livers were then washed with ice cold saline, chopped and a homogenate solution was prepared in tris Hcl buffer (pH 7.4). The homogenate solution was centrifuged at 10000 rpm for 10 minutes at 4°C and the homogenate solution was used for the estimation of malondialdehyde (MDA)[16] and total protein[17], while the supernant was used for estimation of glutathione (GSH)[18].

Histopathological studies

One animal from the treated groups showing maximal activity as indicted by improved biochemical parameters from each test, positive control, hepatotoxin and control groups were elected to undergo histopthological studies. The animals were sacrificed, and abdomen was open to remove the liver. A portion of liver tissue was preserved in 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopy of the liver cells was done. [11, 12]

Isolation of rat hepatocyte

The hepatocytes were isolated by recalculating enzymatic perfusion technique [13]. The abdomen of the rat was opened under ether anesthesia and 0.2 ml of 0.2%w/v heparin in 0.9%w/v NaCl was injected into the tail vein to prevent blood clotting. A midline incision was made and the portal vein was cannulated with a needle fitted with a teflon catheter. The Teflon catheter was tied in place and the needle was removed. The inferior vena cava was cut below the renal vein. The liver was perused in-situ through the portal vein using calcium free HBSS (pH 7.4) containing 1% bovine serum albumin and 0.5mM EGTA [19,20]. The initial flow rate was 30 ml/min and aeration was carried out with 95% O_2 / 5% CO_2 to pH 7.4 at 37° C. After ten minutes of perfusion when liver was completely bleached and freed from the blood, the inferior vena cava was tied off above the renal vein and the thorax portion of the superior vena cava was cannulated. The perfusion of the liver was done for 10 min with the calcium free Hank's buffer (100 ml) (containing additionally 0.075% collagenase and 4 mM $CaCl_2$) is recirculated. After 10-15 min of perfusion, the liver was transferred to a beaker containing phosphate buffer (50 ml) and gently dispersed with two forceps. The crude cell suspensions were then rotated in rotor for 10 min. The cell suspension was then cooled in ice and filtered gently through cotton gauze into centrifuge tubes.

The preparation is centrifuged at 8000 rpm for 1 min. the supernatant was removed and the loosely packed pellet of cells was gently resuspended in phosphate buffer. The washing procedure was repeated 5 times.

Hepatocyte viability tests: (Trypan blue exclusion test)
The isolated cells were subjected to viability test by putting them into trypan blue solution (0.2%). The unstained viable cells were easily distinguished from the blue stained damaged cells. The percent protection was calculated by comparing with damaged group as judged by the number of viable cells.

Statistical analysis
The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). P values <0.05 were considered significant using Microsoft excel 2003 edition.

Results and Discussion

Ethanol administration resulted in significant elevation of AST, ALT, ALP, triglyceride, direct bilirubin and total bilirubin levels (Table 1) compared to normal control group. Treatment with silymarin (200mg/kg) and *S.officinarum* juice (0.75ml/100gm) significantly prevented the biochemical changes induced by ethanol.

Table No. 1: Effect of *S.officinarum* juice on serum enzymes in ethanol induced hepatotoxicity.

Parameter	Normal control	Ethanol Control	Ethanol+ silymarin (p.o) (100mg/kg)	Ethanol+ *S.officinarum* Juice (p.o) (0.75ml/100gm)
Alkaline phosphate (IU/L)	11.78±2.72	102.28±10.13 #	50.42±8.25 * *	61.73±10.34 * *
SGPT (IU/L)	30.5 ±5.37	95 ±9.57 #	58 ±7.83 * *	67 ±7.04 * *
SGOT (IU/L)	42±4.49	127 ±8.96 #	62 ± 7.25 *	68.5 ± 7.5 *
Total bilirubin (mg/dl)	0.295 ±0.067	1.22 ±0.1 #	0.67 ±0.017 *	0.76 ±0.063 *
Directbilirubin (mg/dl)	0.094 ±0.014	0.30 ±0.010 #	0.16 ±0.014 *	0.23 ±0.025 *
Triglycerides (mg/dl)	92.05 ±12.04	212.31± 3.27 #	137.67± 7.18 *	153.17 ±6.5 *

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). P values <0.05 were considered significant and highly significant P<0.001; # significantly different from normal control P ≤ 0.001; * * significantly different from ethanol control P ≤ 0.05; * significantly different from ethanol control P ≤ 0.001

In order to probe the possible mechanism by which *S.officinarum* prevents hepatic damage caused by ethanol, investigation on levels of total protein, MDA and glutathione were carried out [21]. MDA were found to be elevated after the administration of ethanol which was significantly

reversed by *S.officinarum*. There was a significant rise in total protein and GSH content of liver after treatment with *S.officinarum*. The effects of *S.officinarum* were comparable to that of silymarin. (Table 2)

Table No. 2: Effect of *S.officinarum* juice on total protein, malondialdehyde level and reduced glutathione level in ethanol induced liver damage.

Parameter	Normal control	Ethanol Control	Ethanol + silymarin (p.o) (100mg/kg)	Ethanol + *S.officinarum* Juice (p.o) (0.75ml/100gm)
Protein	7.48 ±0.681	4.44 ±0.205 # #	5.53 ± 0.158 * *	5.12 ±0.143 * *
MDA	0.265 ±0.028	1.05 ±0.059 #	0.40 ± 0.015 *	0.46 ± 0.025 *
GHS	6.07 ±0.541	4.26 ±0.356 # #	5.93 ±0.237 * *	5.08 ±0.201 * *

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). P values <0.05 were considered significant and highly significant P<0.001. # # Significantly different from normal control $P \leq 0.05$; # Significantly different from normal control $P \leq 0.001$; **Significantly different from ethanol control $P \leq 0.05$; *Significantly different from ethanol control $P \leq 0.001$.

Administration of ethanol significantly increased liver weight and volume as compared to normal control. Rats treated with silymarin and *S.officinarum* juice showed significant decrease in wet-liver weight and volume compared to ethanol control group. In thiopentone induced sleeping time studies, *S.officinarum* also increased onset time (in seconds) and decrease duration **(**in minutes) as compared to ethanol control. (Table 3)

Table No.3: Effect of *S.officinarum* juice on liver weight, liver volume, and thiopentone induces sleeping time (second) and (minutes) in ethanol induced liver damage.

Parameter	Normal control	Ethanol Control	Ethanol+ silymarin (p.o)(100mg/kg)	Ethanol+*S.officinarum* Juice (p.o) (0.75ml/100gm)
Liver weight/100gm	3.32 ±0.094	4.94 ±0.091 #	3.48 ±0.118 *	3.92 ± 0.082 *
Liver volume/100gm	3.34±0.108	5.02 ±0.099 #	3.64 ±0.134 *	4.04 ± 0.069 *
Thiopentone induce Sleeping time (sec)	178.7±11.71	117.9 ±7.14 # #	171.95 ± 5.40 *	160.35 ±5.92 *
Thiopentone induce Sleeping time (min)	85.7 ±3.074	147.4 ±9.15 #	99.73 ±5.31 *	105.9 ± 4.19 *

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA). P values <0.05 were considered significant and highly significant P<0.001.; # # Significantly different from normal control $P \leq 0.05$; # Significantly different from normal control $P \leq 0.001$; * Significantly different from ethanol control $P \leq 0.001$

In control animals, liver sections showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and normal liver parenchymal cells. Ethanol (3.76 gm/kg/day, p.o) induced hepatic injury produced liver cell necrosis. Juice of *S.officinarum* (0.75ml/100gm., p.o. for 8 to 18 days) treated liver showed diffuse areas of liver cells necrosis and isolated liver cells and focal areas of liver cells showed evidence of regeneration. Silymarin (200mg/kg., p.o. for 8 to 18 days) treated liver histopathology also showed diffuse areas of liver cells necrosis and focal areas of liver cells regeneration.(Figure1-4)

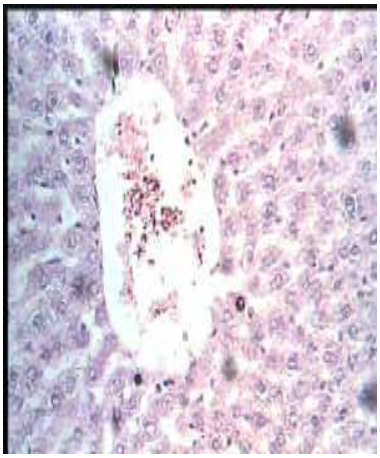

Figure 1: Normal control

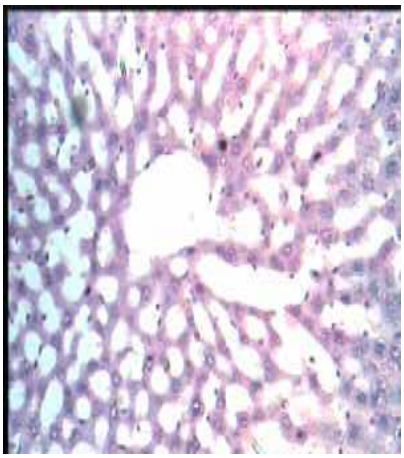

Figure 2: Ethanol control

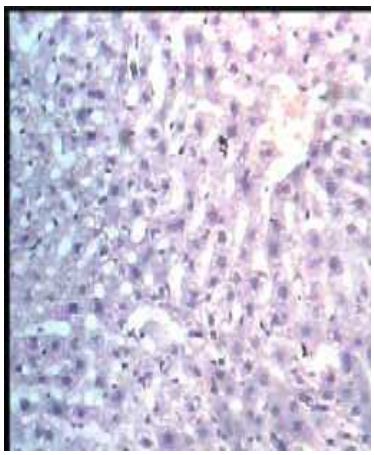

Figure 3: Ethanol + Silymarin (100mg/kg)

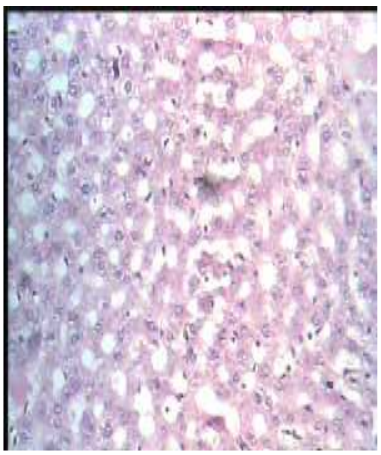

Figure 4: Ethanol + *S.officinarum* (0.75ml/100gm)

In the present in vitro study, ethanol induced cells damage. Ethanol (20% of 3.76gm/kg., p.o. for 18 days) significantly decreased percentage viability of cells as compared to normal control. Treatment with silymarin (100mg/kg., p.o. for 9 to 18 days) significantly increased percentage viability of cells as compared to ethanol control. Treatment with *S.officinarum* juice (0.75ml/100gm., p.o. for 9 to 18 days) significantly increased percentage viability of hepatocytes (Table 4 and Figure 5 to 8).

Table No. 4: Effect of *S.officinarum* juice on percentage of viable cells in ethanol induced hepatocytes damage

Parameter	Normal control	Ethanol Control	Ethanol + Silymarin (100mg/kg) treated	Ethanol + *S.officinarum* Juice (0.75ml/100gm)
% of viable cells	60.87 ± 0.322	25.90 ± 0.962 #	59.12 ± 0.6 *	55.09 ± 0.627 *

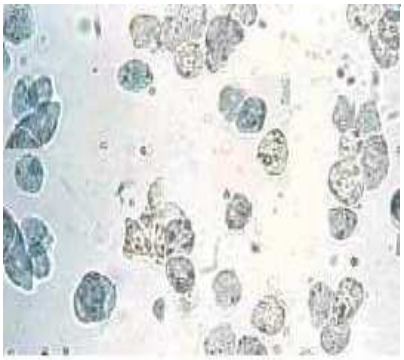

Figure 5: Normal control

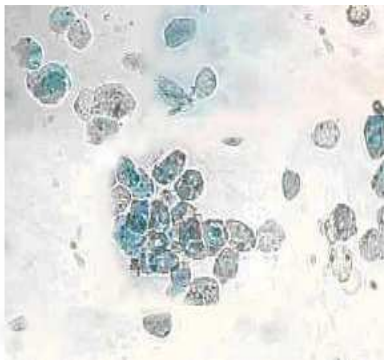

Figure 6: Ethanol control

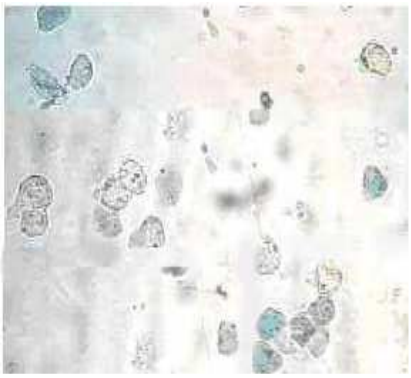

Figure 7: Ethanol + Silymarin (100mg/kg)

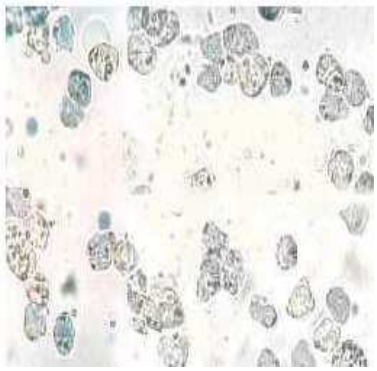

Figure 8: Ethanol + *S.officinarum* (0.75ml/100gm)

Conclusion

Saccharum officinarum (0.75ml/100gm., p.o.) significantly decreased SGPT, SGOT, ALP, triglyceride and bilirubin in the animals treated with ethanol. It is also significantly decreased MDA and protein levels and increased GSH levels confirming its antioxidant activity in the animals treated with ethanol. Similar results were obtained with standard drug (silymarin).

Saccharum officinarum (0.75ml/100gm., p.o.) were also significantly decrease liver weight and volume and it is also increased thiopentone induce sleeping time (onset second) and decrease in duration of minutes in the animals treated with ethanol .In vitro model, in addition it also shows increased percentage viability of hepatocytes in cell suspension pretreated with ethanol.

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