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HEPATOPROTECTIVE ACTIVITY OF SACHHARUM OFFICINARUM AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The hepatoprotective activity of juice of *Saccharum officinarum* was investigated against paracetamol (acetaminophen) induced liver damage in rats. Paracetamol (PCM) is analgesic, antipyretics drugs available as an over the counter (OTC) medication which cause hepatotoxicity as higher doses. Paracetamol at 3gm/kg induced liver damage in rats manifested by statically increased serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphates (ALP), total and direct billirubin. The antioxidant parameter malondialdehyde (MDA) level was increase and reduced glutathione (GHS), total protein (TP) level were decrease. The physical parameter of liver weight and volume also increase. Histopatholgical and in vitro (% of cell viability) studies of the experimental animals were also done. The results indicate that the juice of *Saccharum officinarum* protects against PCM-induced hepatotoxicity presumably via antioxidant action.

INTRODUCTION: Many therapeutic agents have injuries effects on the liver and impair liver function leading to liver damage1 and it is also a major reason for the removal of new drugs from clinical development and widespread use2. Paracetamol (PCM) is one of the most commonly used non-narcotic analgesic, antipyretic agents a domain concern over it is that of hepatic toxicity resulting from accidental or deliberate over-dosage. Massive over dosage of PCM is associated clinically with severe central lobular hepatic necrosis and death from liver failure3. Over dosing of paracetamol to rats is reported to decrease their sensitivity to its hepatotoxic effects, which are associated with oxidative stress4. So the studies on antioxidant enzymes, reduced Glutathione (GHS), malondialdehyde (MDA) have been found to be of great importance in the assessment of liver damage. Herbs have become attractive as food that confer a health benefit and as a source of material for the development of drugs. The Saccharum *officinarum* (SO), family Poaceae, it is extensively cultivated in hotter parts throughout India.the plant contains amongst many others flavonoids, alkaloids, amino acids, carbohydrates, starch and vitamins. The use of the plant include as a cardiotonic, expectorant, haemostatic, tonic, diuretic and useful in urinary disorders. The plant has better antioxidant effects. The present study was undertaken to evaluate the hepatoprotective activity of these plants in experimental animal.

MATERIALS AND METHODS:

Chemicals: Paracetamol (PCM), 5', 5'-dithiobis- 2- nitrobenzoic acid (DTNB), GSH, MDA, tris HCl , thiobarbituric acid (TBA), collagenase , trichloroacetic acid (TCA), disodium hydrogen phosphate (Na_2HPO_4), heparin injection, NaCl, KCl, NaOH, glucose, $MgSO_4.7H_2O$.

Animals: Wistar strain albino rats (150-200 g) of either sex maintained under standard husbandry conditions (temperature: 23*2O C, relative humidity: 55*10% and 12 h light and dark cycle) 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 approval of Institute Animal Ethics Committee was obtained.

Paracetamol induced hepatotoxicity[5]: Rats were divided into four groups of six each: control, hepatotoxin, test and positive-control groups. The control group received vehicle at 0, 24 and 48 h orally. The animals in the hepatotoxin group received the vehicle at 0, 24 and 48 h, followed by paracetamol at a dose of 3 g/kg orally. The test group received the first dose of extract at 0 h; the second dose of extract at 24 h; and at 48 h, the third dose of extract followed by a dose of paracetamol. The animals in the positive-control group received the first dose of silymarin (200 mg/kg orally)6 at 0 h; the second dose of silymarin at 24 h; and at 48 h, the third dose of silymarin followed by a dose of paracetamol. After 96 h, blood was collected from all the groups and was allowed to clot, for the separation of serum. Serum was utilized for estimation of SGOT, SGPT, ALKP and TBL by reported methods to assess liver functions.

Assessment of liver functions: Biochemical parameters i.e., serum glutamic oxalacetic transaminases (SGOT), serum glutamic pyruvic transaminase¬7 (SGPT), serum alkaline phosphataseg8 (ALP), serum total bilirubin (STBiI), and serum direct bilirubin9 (SDBiI) which give an idea regarding the functional state of the liver were analyzed according to the reported methods.

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 utilized this purpose. The animals were scarified, and abdomen was open to remove the liver. A portion of liver tissue was preserved in 10% formaldehyde solution for histo-pathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver cells were photographed[10, 11].

Isolation of rat hepatocyte: The hepatocytes were isolated using the method of Seglen P12 by recirculating enzymatic perfusion technique. The abdomen of the rat is opened under ether anesthesia and 0.2 ml of 0.2%w/v heparin in 0.9%w/v NaCl is injected into the tail vein to prevent blood from clotting. A midline incision is made and the portal vein is cannulated with a needle fitted with a Teflon catheter. The Teflon catheter is tied in place and the needle is removed. The inferior vena cava is cut below the renal vein. The liver is perfused in-situ through the portal vein using Calcium free HBSS (pH 7.4) containing 1% bovine serum albumin and 0.5mM EGTA. The initial flow rate is 30 ml/min and aeration is carried out with 95% O2 / 5% CO2 to pH 7.4 at 37° C.

After ten minutes of perfusion when liver is completely bleached and freed from the blood, the inferior vena cava is tied off above the renal vein and the thorax portion of the superior vena cava is cannulated. The perfusion of the liver is done for 10 min with the calcium free Hank's buffer (100 ml) (containing additionally 0.075% collagenase and 4 mM CaCl2) is recirculated. After 10-15 min perfusion, the liver was transferred to a beaker containing phosphate buffer (50 ml) and gently dispersed with two forceps. The crude cells suspensions were then rotated in rotate 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 50 g 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 3-5 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.

RESULTS: Paracetamol administration resulted in significant elevation of AST, ALT, ALP, direct bilirubin and total bilirubin levels (Table 1) and MDA, while total protein and GHS were found to be decreased compared to normal control group (Table 2). Treatment with silymarin (200 mg/kg) and *S. officinarum* juice (0.75 ml/100gm) significantly prevented the biochemical changes induced by paracetamol. Administration of paracetamol 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 paracetamol control group (Table 3).

Table 1: Effect of *S. officinarum* juice on serum enzymes in paracetamol induced hepatotoxicity.

Parameter	Normal control	PCM Control	PCM + silymarin (p. o) (200mg/ kg)	PCM + *S.officinarum* Juice (p.o) (0.75ml/100gm)
Alkaline phosphate (IU/L)	11.78 ± 2.72	99.73 ± 17.84 # #	30.08± 6.76 * *	37.8± 9.32 * *
SGPT (IU/L)	30.5 ± 5.37	158 ± 11.1 #	57± 7.76 *	83± 8.34 *
SGOT (IU/L)	42 ± 4.49	176.5 ± 16.38 #	57.5± 6.39 *	67± 7.55 *
Total bilirubin (mg/dl)	0.295 ± 0.067	1.119 ± 0.028 #	0.498 ± 0.066* *	0.656± 0.114**
Direct bilirubin (mg/dl)	0.094 ± 0.014	0.345 ± 0.094 # #	0.108 ± 0.032* *	0.125 ± 0.017**

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 ≤ 0.05

Significantly different from normal control P ≤ 0.001

* * Significantly different from PCM control P ≤ 0.05

* Significantly different from PCM control P ≤ 0.001

Table 2: Effect of *S.officinarum* juice on total protein, malondialdehyde and reduced glutathione level in paracetamol induced liver damage.

Parameter	Normal control	PCM Control	PCM + silymarin (p.o) (200mg/kg)	PCM + *S. officinarum* Juice (p.o) (0.75ml/100 gm)
Protein (mg/ml)	7.48 ± 0.681	4.24 ± 0.216 # #	5.68 ± 0.223 * *	5.24 ± 0.215 * *
MDA (µg/mg/protein)	0.265 ± 0.028	1.27 ± 0.157 #	0.402 ± 0.051 *	0.483 ± 0.031 *
GHS (µg/mg/protein)	6.07 ± 0.541	4.38 ± 0.512	5.92 ±0.243 * *	5.15 ± 0.147 * *

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 ≤ 0.05

Significantly different from normal control P ≤ 0.001

* * Significantly different from PCM control P ≤ 0.05

* Significantly different from PCM control P ≤ 0.001

Histopathological examination of liver sections of normal control group (Fig. 1) showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein. In the liver sections of the rats intoxicated with paracetamol (Fig. 2), there is disarrangement and degeneration of

normal hepatic cells with intense centrilobular necrosis extending to mid-zone and sinusoidal haemorrhages and dilatation.

Table 3: Effect of *S.officinarum* juice on liver weight and liver volume in paracetamol induced liver damage.

Parameter	Normal control	PCM Control	PCM + silymarin (p. o) (200mg/kg)	PCM + *S.officinarum* Juice (p.o) (0.75ml/100gm)
Liver weight/100 gm	3.32 ± 0.094	5.33± 0.144 #	3.6± 0.179 *	3.96± 0.161 *
Liver volume/100gm	3.34± 0.108	5.59± 0.196 #	3.87± 0.162 *	4.21± 0.183 *

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 ≤ 0.05

Significantly different from normal control P ≤ 0.001

* * Significantly different from PCM control P ≤ 0.05

* Significantly different from PCM control P ≤ 0.001

There was chronic inflammatory cell infiltrate in the portal tracts. The liver sections of the rats treated with *S. officinarum* juice and intoxicated with paracetamol (Fig. 3) and rats treated with silymarin and intoxicated with paracetamol (Fig. 4) showed less vacuole formation, reduced sinusoidal dilation, and less disarrangement and degeneration of hepatocytes, indicating marked regenerative activity. The intensity of centrilobular necrosis was less.

In the present in vitro study, paracetamol (3 gm/kg., p. o. for 3 days) induced liver damage.

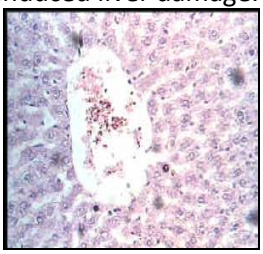

Figure 1: Normal control

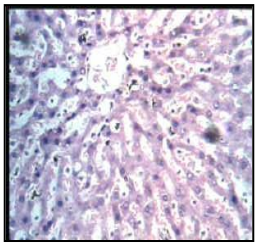

Figure 2: PCM control

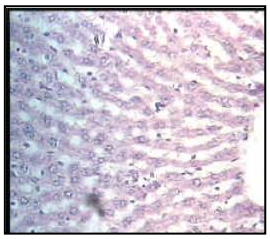

Fig. 3: PCM+Silymarin (200mg/kg)

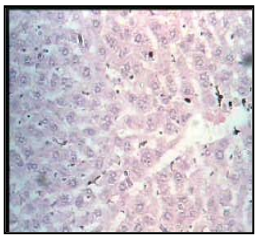

Fig. 4: PCM+*S.officinarum*

Paracetamol significantly decreased % viability of cells as compared to normal control. Treatment with silymarin (200 mg/kg., p. o. for 3 days) significantly increased percentage viability of cells as compared to paracetamol control (Table 4). Treatment with *S. officinarum* juice (0.75 ml/100gm., p. o. for 3 days) significantly increased percentage viability of hepatocytes (Fig. 5).

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

Parameter	Normal control	PCM Control	PCM + Silymarin 200mg/kg) treated	PCM + *S.officinarum* juice (0.75ml/100gm)
% of viable cells	60.87 ± 0.322	28.77 ± 1.095 #	58.28 ± 0.398 *	53.83 ± 0.714 *

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 PCM control P ≤ 0.001

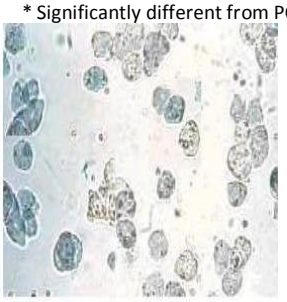

Fig. (5 A)

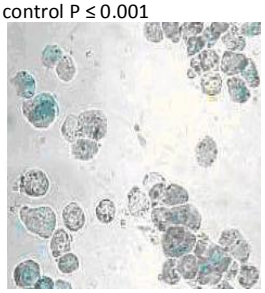

Fig. (5 B)

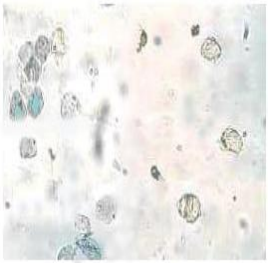

Fig. (5 C)

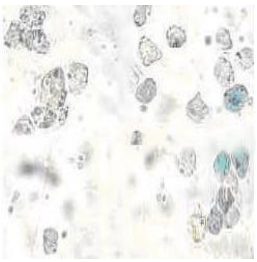

Fig. (5 D)

Figure 5: (A) Normal hepatocytes showed percentage of viability of hepatocytes cells; (B)Paracetamol control shows non-viable cells as compare to normal control; (C) Paracetamol + Siylmarin (200mg/kg) shows more viable cells as compare to carbon tetrachloride control; (D) Carbon tetrachloride + *Saccharum officinarum* (0.75ml/100gm) shows similar viable cells as compare to carbon tetrachloride control

DISCUSSION: Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses; overdoses, however, produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion, but when the conjugation enzymes are saturated, the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative by cytochrome P450 enzyme. The hydroxylamine derivative, a reactive electrophillic agent, reacts non-enzymatically with glutathione and detoxifies. When the hepatic reserves of glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome P450 or depletion of hepatic glutathione is a prerequisite for paracetamol-induced toxicity[13]. Therefore, the hepatoprotective activity of the drug may be due to inhibition of cytochrome P450 or promotion of its glucuronidation or stimulation of hepatic regeneration or activation of the functions of reticuloendothelial systems or inhibition of protein biosynthesis.

In the present study paracetamol caused significant increase in the serum levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phophatase (ALP) and bilirubin. Treatment with *Saccharum officinarum* juice 0.75ml/100gm significantly decreased the levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin. Treatment with silymarin (200mg/kg) significantly decreased the levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP) and bilirubin.

Paracetamol caused a significantly increase the level of malondialdehyde (MDA) and decreased the level of total protein and reduced glutathione in the

liver tissue. In the juice of *S.officinarum* was significantly decrease the level of malondialdehyde (MDA) and increase the level of total protein and reduced glutathione.Paracetamol caused significantly increase wet liver weight and volume. This paracetamol-induced increase in total wet-liver weight and volume was prevented by treatment with *S. officinarum* juice, thus indicating a hepatoprotective effect. Similar hepatoprotection was observed with the pretreatment of *S. officinarum* and silymarin in in-vitro study. Numbers of viable cells were found significantly high in the *Saccharum officinarum* (1mg/1ml) and silymarin (1mg/1ml) pretreated cell suspension as compare to paracetamol control.In histopatological study of liver, animals show central vein and normal liver parenchymal cells. The paracetamol shows centrilobular hepatic necrosis, inflammation and infiltration of lymphocytes. In the treated groups of *S.officinarum* juice (0.75ml/100gm), focal areas of cell necrosis, inflammation and extensive areas of liver cells regeneration. In treatment with standard (Silymarin) shows more specific areas of liver cells regeneration

CONCLUSION: Juice of *Saccharum officinarum* (0.75ml/100gm., p.o.) showed significant hepatoprotective action against paracetamol induced hepatic injury. This activity may be due to its antioxidant property.

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