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HEPATOPROTECTIVE ACTIVITY OF MUSA SAPIENTUM FRUIT PEEL (MSPE) AGAINST STREPTOZOTOCIN – INDUCED TOXICITY IN RATS

S. Sathiya Narayana Murthy[*1], Christilda Felicia Jebakani[1], S. Sundara pandian[1], S. Ponmozhi.[2]

[1]Department of Anatomy, SRM Medical College & Research Centre, Kattankulathur, Kancheepuram District, Tamil Nadu, India, Pin – 603203
[2]Omsakthi Siddha Health Care, Guduvenchery, Kancheepuram District, Tamil Nadu, India, Pin – 603203

***Corresponding author e-mail:** drsnmanat@gmail.com

ABSTRACT

In this study, to assess the Hepatoprotective activity of Acetone extract of Musa sapientum fruit peel (MSPE) against Streptozotocin (STZ) induced hepatotoxicity in rats. Totally Thirty six male albino wistar rats were divided in to 6 groups; Group I received distilled water; animals in the Groups II,III,IV,V are induced single injections STZ 45mg/kg intraperitonialy; Groups IV,V,VI received MSPE in doses of 200,400,800 mg/kg/day respectively. The result obtained in this work was determined the MSPE at the doses 200 and 400 mg/kg/day showed significant hepatoprotective effect by reducing the serum marker enzymes such as Serum Alanine aminotransferase (ALT), Aspartate amino transferase (AST) and Alkaline phosphatase(ALP) and its Histopathological studies further confirmed the hepato protective of MSPE when compared with the STZ induced hepatotoxicity groups. In the conclusions present study findings of Acetone extract of Musa sapientum fruit peel showed significant hepatoprotective activity compared with control groups.

Key Words: Musa sapientum, Streptozotocin induced hepatotoxicity, Alkaline phosphatase, Hepatoprotective activity, Histology of Liver.

INTRODUCTION

Liver diseases especially drug induced hepatoxicity constitute a major cause of morbidity and moratality in man.(1) Streptozotocin is a naturally occurring Nitrosurea with molecular weight of 265 and empirical formula is C14H27NS012.(2) Streptozotocin is a diabetogenic, hepatotoxic, nephrotoxic and causes gastric ulceration.(3) A significant amount of liver damage is induced by lipid peroxidation and other oxidative damages which are caused by the hepatotoxic chemicals.(4,5,6) Diabetes is a chronic disease with a relatively high prevalence in many populations across the world.(7) Diabetes is associated with several structural and functional liver abnormalities that affect glycogen and lipid metabolism.(8,9,10) Excess glycogen deposition, fibrosis, cirrhosis, steatohepatitis and Biliary disease in the liver has been reported in 55 – 80% of diabetic patients.(11) There are many natural products such as plant and traditional medicinal herbs are available for the protective of liver against injury induced by hepatotoxin. More than 600 commercial herbal products with claimed Hepatoprotective role are being sold in all over the world. Around 170 phytoconstituents isolated from 110 plants belonging to 55 families have been reported to show Hepatoprotective role. However, only a small proportion of Hepatoprotective plants as well as formulations used in traditional medicine are pharmacologically evaluated for their safety and efficacy.(12) Musa sapientum which is commonly called banana is a herbaceous plant of the family Musaceae. It is known to have originated from the tropical region of Southern Asia.(13) The fruit of Musa sapientum is traditionally used in diarrhoea (unripe), dysentery, intestinal lesions in ulcerative colitis, diabetes (unripe), in sprue, uremia, nephritis, gout,

hypertension, cardiac disease.[14] Considering its good antioxidant activity and hepatoprotective activity needs to be investigated using a STZ that directly causes toxicity in rats. The goal of this study was to determine the Hepatoprotective effect of Musa sapientum fruit peel in STZ- Induced Liver toxicity in Rats.

MATERIALS AND METHODS

Plant material: Musa sapientum fruits have been collected through extensive field work in various areas in and around SRM University, Tamilnadu, India. The plant and fruit peel has been authenticated and identified by Department of Plant sciences, Bharathidasan university, India.

Preperation of extract: The fruit peels were removed and air dried for 72 hours and then oven dried at 45^{o}C to constant weight. The sample was ground and stored in polythene container for analysis. Dried materials were coarsely ground (2-3 mm) before extraction. Materials were extracted by percolation method using Acetone (80/20 w/w) for 24 h at room temperature. Extracts was filtered and concentrated under reduced pressure at 40°C using a rotary evaporator until a crude solid extracts were obtained which were then freeze-dried for complete solvent removal.

Animal: The study was performed on male albino wistar rats of approximately the same age-group and body weight was 150-200g, housed in ventilated animal house at a temperature of 24 ± 2°C with a 12h light/dark cycle and 60 ± 5% humidity. They were fed with standard Nutrient laboratory animal feed, manufactured S.R. laboratory rodent feed, Bangalore, India. All experiments were performed according to the norms of the Ethical Committee of SRM university, India which is in accordance with the National Guidelines for animal care and use.

Experimental Protocol: Streptozotocin was from SRL Sisco Laboratory USA. All other chemicals are used in this study were of analytical grade and obtained from Thermofischer, Merck, India. Diabetes was induced by intraperitoneal single injection of STZ at a dose of 45 mg/kg body weight. STZ was dissolved in 0.1 M cold sodium citrate buffer, pH 4.5. After 72 hours, animals with fasting blood glucose levels greater than ≥200mg/dl were considered diabetic and then included in this study. Fasting blood glucose was estimated by using one touch glucometer (Accu-Chek) of Roche Diagnostics, Germany. The duration of experiment was 45 days. The 36 male albino rats were randomly divided into 6 groups (6 rats each) as follows: Group 1, healthy control rats received distilled water ; Group 2, diabetic and no treatment was given of beginning of experiment; Group 3 diabetic rats were treated with glibenclaimade(5mg/kg) was given in intragastric tube ; Group 4, diabetic rats were treated with were treated with MSPE(200mg/kg) as in intragastric tube; Group 5, diabetic rats were treated with MSPE(400mg/kg) as in intragastric tube; Group 6 non-diabetic rats MSPE (800mg/kg) was given in intragastric tube. At the end of experiment, blood samples were collected from the retro-orbital plexus and the sera prepared through centrifuging at for 15 minutes at 30°C. Serum biomarkers of liver function including Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), and Total Proteins(TP) were measured using Commercially Available Kits. ALT and AST were measured to determine the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. ALP were measured to assess biliary function.

Histopathological Studies: The liver was collected and immediately fixed in 10% Formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene and embedded in paraffin. Sections (4-5 µ) were prepared and then stained with Hematoxylin and Eosin (H & E) for photomicroscopic observations.

Statistical Analysis: The values are presented as means ± SD. Differences between group means were estimated using a two pair T test compare with the control group. Results were considered statistically significant when $p < 0.05$.

RESULTS

Hepatoprotective effect of Musa sapientum fruit peel extracts(MSPE) Streptozotocin induced hepatotoxicity in rats have been showed in Table I. Streptozotocin injected as single dose intraperitonialy ,end of the study caused marked hepatotoxicity showing significant ($p < 0.001$) increase in serum AST (180.33%), ALT(163.8%), ALP(132.57%) and decrese in TP (3.75%) as compared to normal control animals. The acetone extract of Musa sapientum fruit peel treated rats differed from normal control rats by an elevated concentration of serum AST (25 and 3.37%), ALT(16.08 and 10.83%) , ALP (3.5 and 8%) at doses 200 and 400 mg/kg/day, intragastric tube respectively. These parameters were found to be statistically significantly different as compared to normal rats (Table I).The effect was dose-dependent which indicated activation of the

regeneration process. A single dose of STZ caused toxicity in the liver. Acetone extracts MSPE at dose 200&400mg/kg/day exhibited maximum protection in the liver (Figure1). The histological changes associated with the hepatoprotective activity in two dosages of extracts basically supported the measuring of the serum enzyme activities. Non diabetic rat cells, shows sinusoidal spaces, and well central vein and Hepatic chords which shows normal cellular architecture, which received citrate buffer only (Figure 1A). However, STZ administration caused classical damage in the rat liver , as demonstrated by severe apoptosis, hepatocyte necrosis, inflammatory cells infiltration and sinusoidal dilation (Figure 1B). Glibenclaimade administered diabetic rats shows normal lobular architecture and slight neutrophil infiltration almost comprable to normal (Figure 1C). The administration MSPE at dose of 200 and 400 could largely rescue the severity of STZ- induced liver intoxication, the higher dose (400 mg/kg) were most effective (Figure 1D-E).

DISCUSSION

Assessment of liver function can be made by estimating the activities of serum biomarker enzymes such as ALT, AST, ALP which are originally present in higher concentration in the cytoplasm of hepatocytes. The increased levels of AST,ALT, ALP are conventional indicators of hepatocellular necrosis caused by oxidative stress in diabetes.[15] In the present study, there was a significant rise in serum biomarkers levels such as ALT,AST,ALP in diabetic rats which could be related to excessive accumulation aminoacids in diabetes.[16] In our study Administration of fraction at the dose of 200 and 400 mg/kg significantly decreased the AST, ALT and ALP towards its normal, indicating the acetone extract of MSPE preserved the structural integraity of the hepatocellular membrane and liver cell damage caused by oxidative stress associated with diabetes, which was confirmed by histopathological studies.

CONCLUSION

Acetone extracts of Musa sapientum fruit peel posses flavonoids having antioxidant and free radicals scavenging property. It reduces the oxidative stress caused by diabetes. Musa sapientum fruit peel extract shows significantly reducing elevated serum biomarker levels in the body and shows ameliorate activity against streptozotocin toxicity in rats.

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TABLE 1: Effect of Musa sapientum fruit peel extract (200,400,800mg/kg/day) p.o for 45 days in rats ,and shows serum biomarker enzyme levels of experimental groups

GROUPS	ALT(IU/dl)	AST(IU/dl)	ALP(IU/dl)	TP(g/dl)
Non diabetic normal	61.25±1.03	67.33±6.6	77.33±8.03	7.60±0.05
STZ induced diabetes	225.06±3.50(163.8)***	247.66±2.03(180.33)***	209.90±1.15(132.57)***	3.85±0.31
STZ induced treated with glibenglaimade5mg/kg/day p.o	101.02±1.29(39.77)***	96.60±9.6(29.27)***	94.60±1.39(17.27)**	6.73±0.35
STZ induced treated with MSPE 200mg/kg/day p.o	77.33±3.99(16.08)**	92.33±3.37(25)**	80.83±1.63(3.5)*	5.85±0.31
STZ induced treated with MSPE 400mg/kg/day p.o	72.08±2.11(10.83)**	72.16±1.73(4.83)*	69.33±1.28(8)*	6.15±0.33
Nondiabetic treated with MSPE 800mg/kg/day p.o	57.16±1.46(4.09)	56.90±8.9(10.43)*	59.83±1.73(17.5)*	7.45±0.13

Values are Mean ± SD(n=6).Data for normal animals are considred as base line data; percentage increases (in parantheses) is calculated with reference to normal control * $p \leq 0.05$ versus control group, ** $p \leq 0.01$ versus control group,*** $p \leq 0.001$ versus control group.ALT = Alanine Aminotransferase ,AST = Aspartate Amino transferase, ALP = Alkaline phosphatase ,TP = Total protein, SD = Standard deviation.

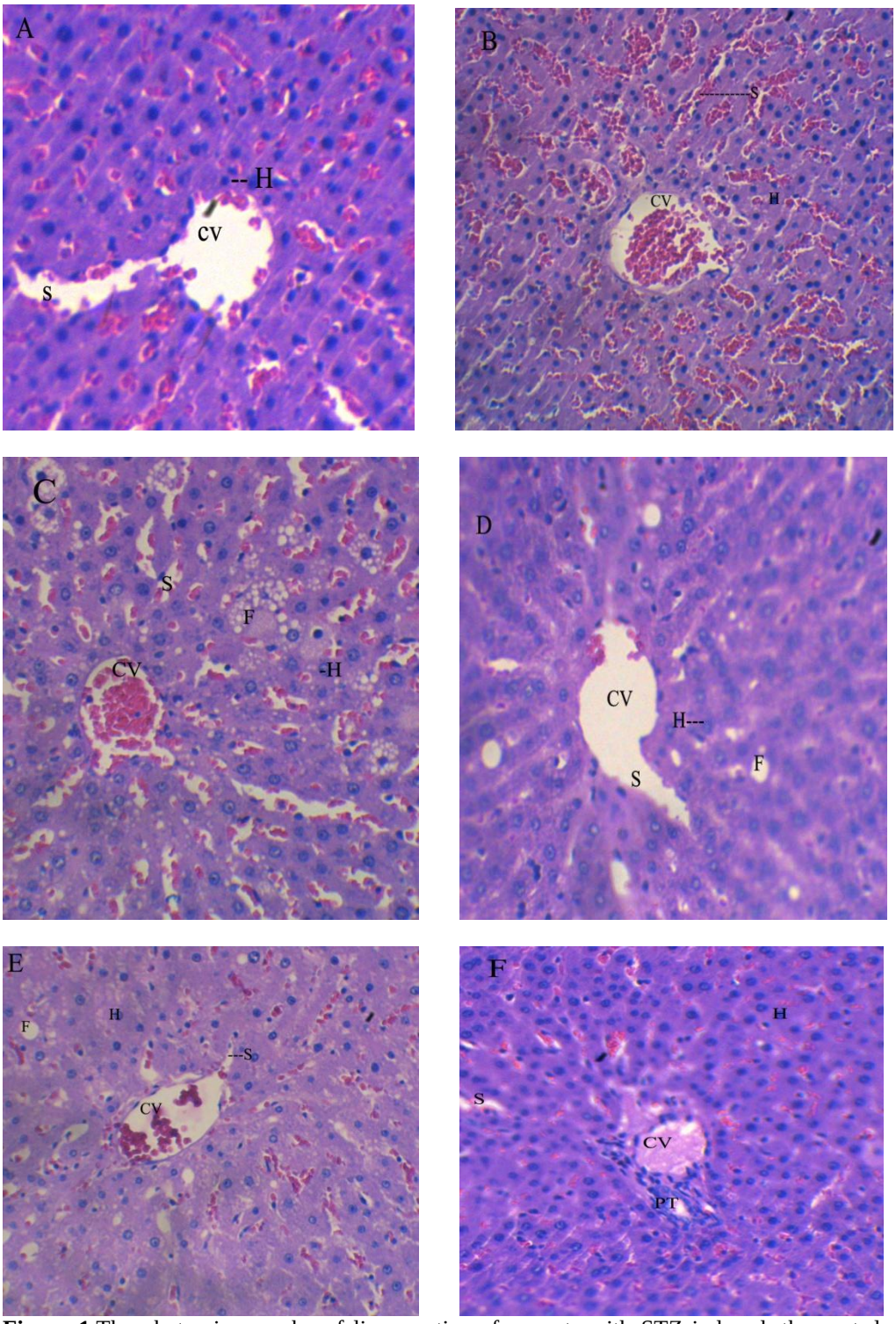

Figure 1. The photomicrography of liver sections from rats with STZ induced, the post doses of MSPE at 200, 400,and 800mg/kg/day, compare with glibenclaimade 5mg/kg/day for 45 days vehicle. Liver sections stained H&E ,A-normal group ,B-STZ induced ,C-STZ induced diabetic with glibenclaimade 5mg/kg/day, D-STZ induced diabetic with MSPE 200mg/kg/day, E-STZ induced diabetic with MSPE 400mg/kg/day, F-MSPE 800mg/kg/day.
Figure legends: Cv-Central Vein, S-Sinusoids, H-Hepatic Cell, F- Fat Cell.

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