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Screening Of Ethanolic Extract Of Diospyros Ebenum For Antidiabetic And Hepatoprotective Effects

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ABSTRACT

Hepatoprotective Effect of Ethanolic extract of Diospyros Ebenum (EDE) in streptozotocin (STZ) 45mg/kg body weight induced diabetic rats was studied. Oral administration of Ethanolic extract of Diospyros Ebenum (EDE) to diabetic induced rats at a dose of 500 mg/kg body weight resulted in significant reduction of elevated blood glucose and hepatic transaminase enzyme levels, at different treatment period (0th day, 21st day, and45th day) which also showed the structural changes in hepatic architecture of STZ induced diabetic rats. The EDE treated diabetic rats were significantly recovered from hepatotoxicity , by analyzing various physical, biochemical parameters like body weight, fasting plasma glucose levels, Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and Alkaline phosphatase (ALP) levels. Further the histopathology results of EDE treated rats also confirmed the significant recovery of liver damage. Our study confirmed the therapeutic hepatoprotective effect of EDE in diabetic treated rats.

Keywords: Diospyros Ebenum, Hepatoprotective, Histopathology, Streptozotocin, Hepatic enzymes, Hepatic cytoarchitecture.

INTRODUCTION

Diabetes mellitus is a serious metabolic disorder with microvascular and macrovascular complications that results in significant morbidity and mortality(1). The prevalence of diabetes worldwide was estimated to rise to 366 million in 2030(2). Diabetes associated with several structural and functional liver abnormalities that affect glycogen and lipid metabolism (3). A significant amount of liver damage is induced by lipid peroxidation and other oxidative damages caused by hepatotoxic chemicals (4). India has a great ancient heritage of traditional medicine, alternative medicines particularly herbal drugs, were prescribed for the treatment of diabetes and its complications, due to their effectiveness, safety profile, affordability, bioavailabilty and less toxic or adverse side effects. World health organization also recommended the evaluation of traditional plant treatment for diabetes.(5) Therefore, investigation of therapeutic drugs from plant medicines became the need of the hour, and also to prove their efficacy and safety in the preclinical experiments or techniques, before prescribed for human beings. According to Ayurveda, India's traditional pharmacopoeia, Diospyros Ebenum, is a perennial weed which is

pungent, bitter, fragrant, appetizer, antihelmentic, antipyretic and alexiteric.(6) Anti-diabetic and hepatoprotective activity of various extracts of Diospyros Ebenum have been reported by many researchers, but the histological study of such effects, at tissue level not reported elsewhere, so in our study we made an attempt to assess hepatoprotective effect of Ethanolic extract of Diospyros Ebenum on histopathology of liver tissue of STZ induced diabetic rats.

MATERIALS AND METHODS

Plant collection and Authentication Diospyros Ebenum was collected from Agumbe, Western ghats, Karnataka, India and authenticated.

Preparation of extract

The whole plant of Diospyros Ebenum was washed with tap water, air dried, and grinded in a mechanical blender. The dried powder (100 g) of Diospyros Ebenum was extracted with ethanol in a soxhlet extractor and the resultant extract was concentrated in a rotary vaccum evaporator, the concentrated dark extract stored in air tight container.

Animals

Adult male albino wistar rats (aged 10 weeks, weighing 150-200 g) approximately were acclimatized and housed in the central animal house. All animals werekept in 12:12 hr light: dark cycle, at a room temperature of 22±2˚C. Rats were fed with standard rat pellet supplied by Provimi animal nutrition India ltd, Bangalore, India, were also allowed free access to water. Animal experimentation were carried out under the supervision of on duty veterinary medical officer in accordance to the ethical norms approved by the Institutional animal ethical committee (IAEC

Experimental diabetes induction

Animals were fasted overnight and diabetes was induced by single intraperitoneal injection of streptozotocin (45mg/kg body weight) prepared in 0.1 M Citrate buffer at pH 4.57. To overcome drug induced hypoglycemia, animals were allowed to drink 5% glucose solution overnight. Citrate buffer alone injected to control rats. After 72 hours of STZ injection, (taken as 0th day) fasting blood glucose levels of each animal were analyzed. Animals with fasting blood glucose levels > 200 mg/dl were considered as diabetic and taken for the study.

Experimental Design

The rats were randomly divided into 5 groups of 6 rats in each group.

Group I : Normal control rats Group II : Diabetic control rats.

Group III : Diabetic rats treated with Glibenclamide (2.5 mg/kg body weight/rat/day) for 45 days, via Oral gavage.

Group IV : Diabetic rats administered with ethanolic extract of Diospyros Ebenum (200 mg/kg body weight/rat/day) for 45 days, via Oral gavage.

Group V : Diabetic rats treated with ethanolic extract of Diospyros Ebenum (400 mg/kg body weight/rat/day) for 45 days, via oral gavage.

Body weight, fasting plasma glucose levels and SGOT, SGPT, ALP levels were measured on 0th day, 21st day and 45th day. plasma glucose was determined by ortho toludine reagent method 8. Blood

collected from the retro-orbital plexuses of the rats of all groups, under lightether anesthesia, serum then separated from the whole blood, were analyzed for SGOT, SGPT and ALP levels by using a semi automatic biochemical analyzer with commercially available biochemical kits.

Collection of tissue samples

After 45 days of experiment, animals were sacrificed, following the guidelines of animal ethical committee. The liver tissues were excised and fixed in 10% neutral buffered formalin (NBF) solution for histological analysis.

Histological preparation of liver tissue

The fixed liver tissues were sectioned with Leica rotary microtome to produce serial sections of 5µ thickness. Liver sections were stained with routine Hematoxylin and Eosin (H&E) stains. The stained slides were then photomicrographed with APCAM-5 USB 2 digital camera attached to a computer monitor, supplied by ADELTAVISION OPTEC India microscope Ltd.

Statistical Analysis

Results were expressed as Mean ± S.E.M and the data were tested by one way analysis of variance (ANOVA) followed by the student-Newman-Keuls post-hoc test using the software "Graphpad Instat". The $p<0.05$ were considered as statistically significant.

RESULTS

Administration of STZ results in a significant increase in plasma glucose level, with the reduction in body weight, also diabetic induced rats showed a significant increase in the levels of SGOT, SGPT, and ALP. However, after the treatment of diabetic rats with
400 mg/kg/ b.w of EDE for 45 days ,the plasma glucose levels decreased significantly ($p<0.001$),with simultaneous increase in body weight ,also the elevated SGOT,SGPT,ALP levels were significantly lowered ($p<0.001$), when compared with the levels of diabetic control and diabetic glibenclamide treated groups of rats. However, the normal rats treated with EDE 500 mg/kg b.w. also showed normal levels of plasma glucose, SGOT, SGPT, ALP levels, when compared with the control group of rats fed with distilled water, as shown in Table 1, Table 2 and Table 3.

Table 1 Effect of EDE on body weight in normal &experimental rats

Groups	**Change in Body weight (gm)**		
	0 day	21st day	45th day
Group I	177±2.58	181.66±2.41	192.16±2.98
Group II	180.66±2.13**	154.83±1.47**	123.33±1.96**
Group III	175.33±2.15#	179.33±2.44**	183.16±1.97**
Group IV	178.16±1.60	186.50±2.14**	180.66±1.60**

GroupV	176±2.51#	180.66±2.21**	184.50±1.45**

Results are expressed as mean ±SEM; n=6; **=p<0.001 and# =notsignificant

Table 2 Effect of EDE on plasma glucose values in normal & experimental rats

Groups	**Plasma glucose levels in mg/dl**		
	0 day	21st day	45th day
Group I	98.16±2.22	96.33±1.76	95.5±2.12
GroupII	271.33±8.80**	330±11.07**	371.83±11.85**
GroupIII	266.66±8.53#	197±7.10**	120.5±2.95**
Group IV	89.50±0.76	89.5±1.47**	87.16±0.70**
GroupV	264.50±7.02#	192.5±10.67**	117.5±2.39**

Results are expressed as mean ±SEM;n=6; **=p<0.001 and # =notsignificant.

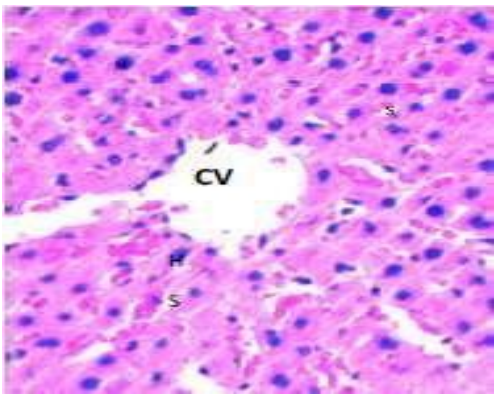
Table 3 Effect of EDE on SGOT, SGPT and ALP levels in normal & experimental rats

Groups	**SGOT (IU/L)**		**SGPT (IU/L)**		**ALP (IU/L)**	
	0 day	**45th day**	**0 day**	**45th day**	**0 day**	**45th day**
Group I	62.58±1.43	61.25±1.03	65.5±0.67	67.33±0.66	77±1.29	77.43±0.80
GroupII	126±1.98*	225±3.50**	171.33±5.07**	247.83±2.05**	144.66±1.58**	209±1.15**
GroupIII	130±2.74#	101±1.29**	175±1.93#	95.83±1.07*	145.33±1.28#	94.16±1.49**
GroupIV	62.33±1.92	60.33±0.61**	67.16±0.16	67.83±1.30*	78.83±0.60	78.5±0.99*
Group V	138±1.48#	86.35±3.34**	175±1.91#	74±2.11**	145.16±2.33#	80.16±0.87**

Results are expressed as mean ± SEM; n=6; ** =p<0.001 and # = notsignificant.

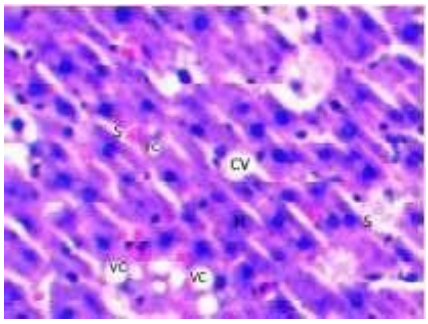
Histopathological study of the liver Examination of the stained sections of the liver of STZ diabetic rats revealed necrotic changes including nucleus and cytoplasmic vacuolation, hepatocytes and sinusoids fragmentation, vascular congestion of the central vein and fatty degeneration (Fig.1.B).

The normal control group (Fig.1.A) and normal treated group (Fig.1.D) showed normal cytoarchitecture of liver tissue with clearly defined hepatocytes around the central vein and well arranged sinusoids between the hepatic plate of cells. Diabetic rats treated with EDE and with glibenclamide, also showed the normal restorationof liver cytoarchitecture (Fig.1.E &C) which was almost similar to control group of rats.



A

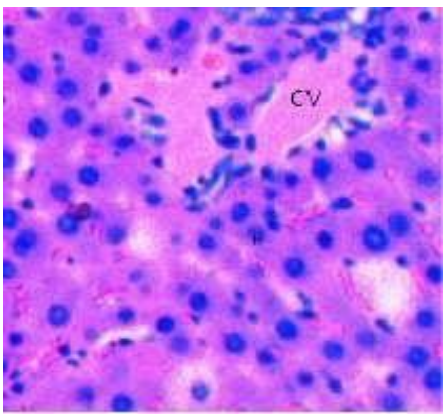
Figure 1 .A Photomicrograph of liver of normal control rat shows clear central vein, well arranged hepatocytes and sinusoids. (H& E magnification X100)



B

Figure 1B

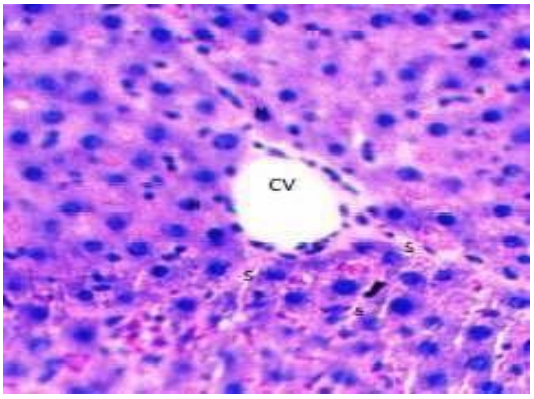
Photomicrograph of liver of STZ induced diabetic rat shows congested central vein, fatty degeneration and cytoplasmic vacuolation. (H& E magnification X100)



C

Figure 1C

Photomicrograph of liver of diabetic rat treated with Glibenclamide (5mg/kg b.w) shows restoration of hepatocytes structure, clear sinusoids and reduction in fatty degeneration. (H& E magnification X100)

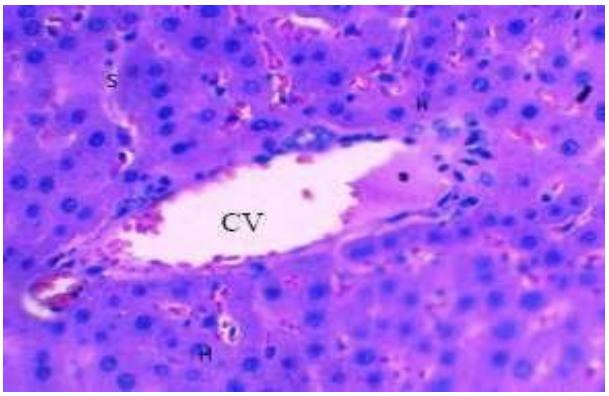


D

Figure 1D

Photomicrograph of liver of diabetic rats treated with EDE (200mg/kg b.w) shows wellarranged

hepatocytes in between sinusoids, with clear central vein. (H& E magnification X100)



E

Figure 1E

Photomicrograph of liver of diabetic rat treated with EDE (400mg/kg b.w.) shows restoration of hepatocytes structure to near normal, still little congestion of central vein seen. (H& E magnification X100)

DISCUSSION

Diabetes mellitus is an endocrine, metabolic disorder in which the homeostasis of carbohydrate and lipid metabolism is improperly regulated by the pancreatic hormone, insulin, ultimately resulting in increased blood glucose(9). Treatment of diabetes mellitus with oral hypoglycemic agents like sulfonylurea & biguanide is associated with serious side effects (10). Traditional herbal drugs have a long history of use and are gaining importance due to its safetyness than synthetic drugs(11). In our study, diabetes was induced in rats by single intraperitoneal injection of STZ at a dose of 45 mg/kg b.w. and the histological basis of hepatoprotective activity of EDE 400 mg/kg b.w. was determined.

Diabetes mellitus is associated with progressive metabolic derangement, worsening glycemic control, and morphological changes in the liver, pancreas and other organs(12,13). Liver enzymes SGOT, SGPT, ALP are present in high concentration in the normal hepatocytes of the liver and these enzymes are leaked into the circulation as a result of damage to cell membrane of hepatocytes(14). The fasting plasma glucose levels were significantly increased in STZ induced diabetic rats, which was significantly ($p<0.001$) reduced by 45 days of treatment with EDE (Table 2). In STZ induced diabetic rats, EDE treatment significantly ($p<0.001$) increased the body weight (Table 1). These results showed that the decreased plasma glucose levels may be correlated with decreased gluconeogenic activity(15) which may be the reason for an increase in body weight in EDE and glibenclamide treated diabetic rats . The elevated levels of SGOT, SGPT in serum are an indication of damaged liver tissue(17). Administration of EDE improves the liver function by decreasing the levels of SGOT, SGPT in diabetic treated rats, indicating its hepatoprotective effect. ALP acts as a marker of biliary function(17). Reduction in ALP levels in EDE treated diabetic rats further validates its hepatoprotective effect. Treatment of normal rats with EDE maintained the levels of hepatic enzymes thereby showing its non-toxic nature.

Further histopathological examination of liver sections of STZ diabetic rats showed marked hepatocyte necrosis, fatty degeneration, and extensive vacuolization and distorted liver structure. Treatment with EDE restored the normal architecture of liver tissue in STZ diabetic rats, thereby proving its hepatoprotective role. No changes were found in the liver histopathology of normal rats treated with EDE, indicating its non-toxic nature. Treatment with glibenclamide also restored the mere normal architecture of liver tissue in STZ diabetic rats, but showed the presence of vascular congestion of the central vein and few hepatocyte nuclei vacuolization.

Conclusion:

The Ethanolic extract of Diospyros Ebenum significantly exhibited its hepatoprotective and antidiabetic effect histologically. The results of the present study will have a profound impact in the treatment of diabetes mellitus and its complications. Further studies on histochemical and immune histochemistry are in process to confirm the same**.**

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