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Hepatoprotactive activity of Aqueous Extract of *Syzygium cumini* Seed on Streptozotocin Induced Diabetes in Rats

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Abstract

The effects of aqueous seed extract of *Syzygium cumini (S.C)* on hepatoprotection in streptozotocin (STZ) – induced diabetic rats were investigated. G-A serves as control, G-B serves diabetic control, G-C administered standard oral hypoglycemic drug, G-D and E administered two graded doses of seed extract. STZ was administered to rats after 72 hours blood glucose was estimated. Rats showed more than 200mg/dl glucose level considered in to diabetic rats. The selected diabetic rats were divided in to G-B, C, D and E. The G-B considered diabetic control group and G-C, D and E groups given their respective drugs for 120 days. On 120th day blood was collected from the all the groups and liver enzymes like AST, ALT, ALP, GGT were estimated by using auto-analyzer. Study results showed increased liver enzymes in G-B compared to all the groups. Standard and *S.C* 500mg/kg groups showed significant decrease in liver enzymes compared to other groups. Low dose of seed extract had less protective effect compared to high dose. From the study results, it can conclude that administration aqueous extract of *S.C* seed powder showed liver protective effect. There is requirement of more studies to isolate active compound form the seed powder.

Keywords: Diabetes, Glibenclamide, hepatoprotective, Insulin, Streptozotocin, *Syzygium cumini*,

Introduction

Hepatotoxicity is defined as injury to the liver that is associated with impaired liver function caused by exposure to a drug, toxins, infections, and another non-infectious agent. Hepatotoxic agents can react with

the basic cellular components and consequently induce cell damage, necrosis leads to fibrosis.[1,2] Despite the fact that hepatic problems are responsible for a significant number of liver transplantations and deaths recorded worldwide. The mortality rate is more patients with liver diseases with any other systemic diseases.[3] Hypertension, diabetes, huperlipidemia, cancer along with liver disease required special care and treatment. Liver play major role in the metabolism of endogenous compounds, drugs and other metabolites.[4] Management of patients having liver along with systemic diseases required special attention to manage liver and systemic problem. Administration of synthetic drugs cause development of serious adverse effects to overcome this plant products are drug of choice.[5] *Syzygium cumini* Linn (family Myrtaceae), commonly known as Jaman (Hindi), is a medicinal plant and utilizable species. Common names are Java plum, Black plum, Jambul and Indian Blackberry.[5] Seeds are sweet, astringent to bowels and good for diabetes. As per Unani system of medicine they acts as liver tonic, enriches blood, and strengthens teeth and gums, ringworm infection of the head. Various extracts of fruit and seeds of *Syzygium cumini* were found to have antidiabetic, antiinflammatory, hepatoprotective, antihyperlipidemic, diuretic and antibacterial activities. [6,7,8] In literature details of morphology, phytoconstituents, medicinal properties and uses of *Syzygium cumini* is very sparse therefore, in present study conducted to find the liver protective effect of seed extract in diabetic rats.

Materials and Methods

Animals

Wister Albino male rats weighing of 230-250gm of rats was included in the study. The animals was maintained at temperature of 25 ± 1^0C and provided diet and water ad libitum. The study ethically cleared by Institutional Animal Ethical Committee, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu.[9]

Study design and settings

Rats were breed and maintained in central animal house, department of Pharmacology, Rajah Muthiah Medical College and Hospital, Annamalai University, Tamil Nadu. Total 24 diabetic rats divided in to 4 groups and 6 normal rats kept in control group.

Group-A: Normal control (Normal Saline)
Group-B: Diabetic control (Streptozotocin 45mg/kg/i.p)[10]
Group-C: Diabetic control (Streptozotocin 45mg/kg/i.p/0day) + Glibenclamide (5mg/kg/orally/120 days)[11]

Group-D: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of *Syzygium cumini* seeds (250mg/kg/orally/120 days)

Group-E: Diabetic control (Streptozotocin 45mg/kg/i.p/0day)+ Aqueous extract of *Syzygium cumini* seeds (500mg/kg/orally/120 days)[12]

Collection and preparation of aqueous extract

Syzygium cumini seeds were collected from rural areas of Chidambaram, Tamil Nadu, India. The *S.C* seeds were dried and powdered and a suspension of 100gm in 200ml distilled water was stirred magnetically overnight at room temperature. It was filtered. The filtrate was evaporated to dryness under reduced pressure in a rotary evaporate. The dark brown semi solid extract was stored and used for further study. The seeds were authenticated with the help of botanist at the Department of Botany, Annamalai University.[13]

Procedure

24 experimental animals received freshly prepared solution of Streptozotocin (45mg/kg) in 0.1ml citrate buffer pH 4.5 solution intra-peritoneal route in a volume of 0.1ml/kg. The animals allowed drinking 5% glucose solution over night to overcome the drug induced hypoglycemia. Rats showed blood glucose level 200-300mg/dl after 72hours considered diabetic rats and included in the study.[14] Control rats were administered normal saline and diabetic rats administered standard and test drugs for 120 days. On 120th days blood samples were collected from the retro orbital vein procedure and centrifuged at 4000RPM for 15 min. Serum was collected and used for estimation of liver enzymes (AST, ALT, ALP, GGT) by standard methods.[15,16,17.] Liver was isolated and stored 10% formalin solution. Small part of liver tissue was used to prepare histology slides. The slides were prepared by standard methods.

Statistical analysis

The data analysed by SPSS (0.6 version) to find statistical significant between the groups. ANOVA (Post hoc test) followed by Sheffs t test applied to find statistical significant at 95% confidence interval. P value less than 0.05 considered statically significant.[18]

Results

Control rats showed normal range of liver enzymes but diabetic control group rats showed high levels of liver enzymes. Administration of Standard and *S.C* seed extract 500mg/kg showed significant decrease in LFT compared to other groups. Same model of results observed in all liver enzymes (AST, ALT, ALP and GGT). High dose of plant extract significantly prevent the liver toxicity in diabetic rats. Plant extract and standard drug administered groups showed normal hepatocytes in histopathological observation.

Discussion

It was observed that aqueous seed extract of *S.C* (500mg/kg) showed significant hepatoprotective effect in diabetic rats compared to other groups. According to previous studies seeds of *S.C* contain glycosides, a trace of pale yellow essential oil, fat, resin, albumin, chlorophyll2, an alkaloid- jambosine3, gallic acid, ellagic acid, corilagin and related tannin,3,6-hexahydroxydiphenoylglucose and its isomer 4,6-hexahydroxydiphenoylglucose, 1-galloylglucose, 3-galloylglucose, quercetin and elements such as zinc, chromium, vanadium, potassium and sodium . Unsaponifiable matter of seed fat contains β-sitoterol. The present protective effect may be due to saponins, tannins and flavonoids present in seed extract. Standard oral hypoglycemic drug and high dose of seed extract showed nearly normal levels of liver enzymes. But 250mg/kg do not show significant effect compared to standard and high dose plant extract administered groups. It indicates in low doses plant extract do not show hepatoprotection. This may be due to antioxidant property of seed extract. Antioxidants neutralize the oxidants generated in the liver. Any changes between levels of oxidants and antioxidants cause development of liver damage. Plants having antioxidant effect use full to treat diseases due to oxidative stress. In this study showed *S.C* have liver protection effect in diabetic rats.

Conclusion

Streptozotocin increased the liver enzymes it significantly prevented by aqueous extract of *Syzygium cumini* seed powder (500mg/kg). The results are proved seed powder has liver protection property. More studies required to bring new hepatoprotective drug in to the clinical trials.

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Table-1: Effect of *Syzygium cumini* seed extract on liver enzymes in diabetic rats (MEAN±SEM)

Groups	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)
Group-A	35.50±4.09	27.83±4.71	76.67±6.06	12.67±2.34
Group-B	85.00±10.00*	61.67±10.33*	139.50±3.27*	25.67±2.88*
Group-C	49.17±3.76*,#	45.50±4.64*,#	92.33±3.50*,#	18.67±2.94*,#
Group-D	54.50±13.13*,#,$	46.00±5.48*,#,$	92.17±1.72*,#,$	19.67±3.50*,#,$
Group-E	40.00±2.19*,#,‖	37.33±3.88*,#,‖	83.00±5.51*,#,‖	15.00±2.19*,#,‖

(*P<0.05 significant compared group-A with other groups, #P<0.05 significant compared group-B with other groups, $P<0.05 significant compared group-C with other groups, ‖P<0.05 significant compared group-D with other groups)

Figure-1: Histology of group-A liver

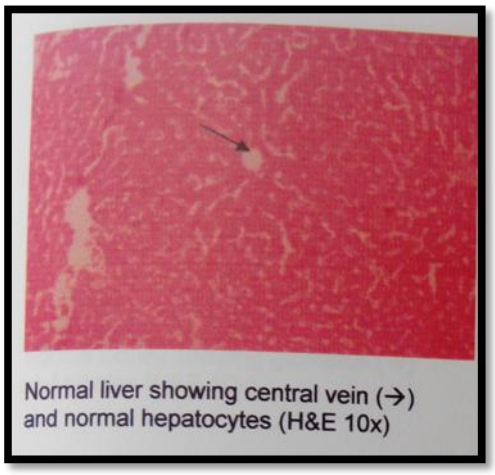

Figure-2: Histology of group-B liver

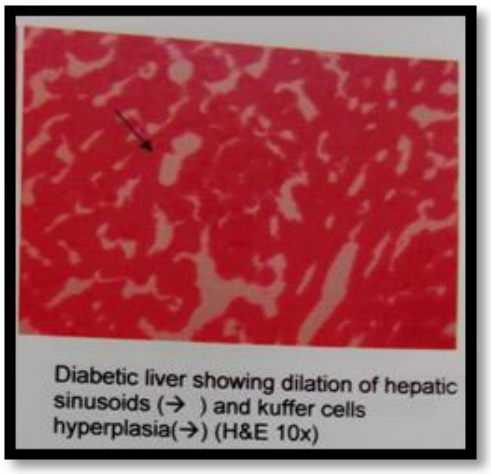

Figure-3: Histology of group-C liver

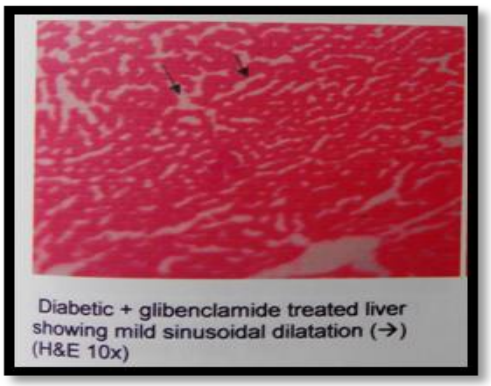

Figure-4: Histology of group-D liver

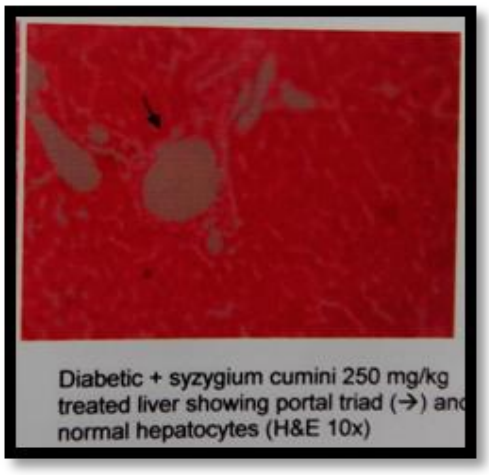

Figure-5: Histology of group-E liver