Available online at www.ijpab.com

ISSN: 2320 – 7051
Int. J. Pure App. Biosci. **2 (4):** 125-131 (2014)
Research Article

INTERNATIONAL JOURNAL OF PURE & APPLIED BIOSCIENCE

Role of *Syzygium cumini* seed-extract on streptozotocin-nicotinamide-induced type-2 diabetes of Albino Rat

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ABSTRACT

Healthy Albino Rats are reared feeding on pulses, gram and bread. After acclimatization these are divided into one normal-control and three experimental groups. Type-2 diabetes mellitus is induced to two of the experimental groups by injecting single dose of streptozotocin (60mg/kg body weight) intraperitonialy 15 minutes after intraperitonial admistration of nicotinamide (120mg/kg body weight). Oral administration of water- extract of Syzygium cumini seeds (400mg/kg body weight) are done to one non-diabetic and one diabetic group of Rats throughout the study period.

Glucose tolerance test of the study animals are done after the study period of 4 weeks. Statistical analysis of data revealed that water-extract of S. cumini seeds can successfully minimize hyperglycemia induced by streptozotocin-nicotinamide administration.

Study of AST (Aspartate Transferase), ALT (Alanine Transferase) and ALP (Alkaline Phosphatase) activities in blood samples and LPO (Lipid Peroxide), GST (Glutathione S-Transferase) and CYP (Cytochrome p450) activities in liver samples of the study animals indicate that no major toxicity arise from the administration of S. cumini seed-extract. Rather, it plays a hepatostimulant role.

Investigation of Urea, Creatinine and Uric Acid levels in blood samples of the study animals lead to a conclusion that no nephrotoxicity occurs from the use of this ethno-medicinal component.

Key words: *Albino rat, diabetes, hepatotoxicity, nephrotoxicity.*

INTRODUCTION

Syzygium cumini L or *Eugenia jambolana* is a very common, large evergreen beautiful tree of Indian subcontinent. It is commonly known as Jamun tree and it belongs to the Myrtaceae plant family. Its fruits are commonly known as kola jamu, java plum; black plum, jambul and Indian blackberry. It grows naturally is clayey loam soil in tropical as well as sub-tropical zones. It is widely cultivated in Haryana as well as the rest of the Indo-Gangetic plains on a large scale. Its habitat starts from Myanmar and extends up to Afghanistan. It is generally cultivated as a roadside avenue tree as well[1]. The fruits of this plant are believed to have hepato-stimulant and hepato-protective properties and various parts of this plant including leaves, bark, roots and seed are used as ethno-medicinal components for diabetes mellitus by the common men in India. In central and lower Assam water-extract of the seeds of this plant is used orally to cure hyperglycemia. Various articles regarding the use of these seeds for the said purpose are though have been published in different journals and periodicals, the information regarding the actual role of this ethno-medicine on the serum glycaemic index is still now very fragmentary. As well as information regarding its toxicological status is not available.

Type-1 and type-2 diabetes mellitus are major health problems amongst different races and classes of people of the society. Though various medicinal and hormonal remedies are available today, people of middle and lower middle classes are still using different plant-based ethno-medicines to control these problems without investigating their actual role on metabolism.

In case of administration of ethno-medicinal components prepared from plant extracts, some problems of intoxications may occur as a single plant or part of it may comprise hundreds of ingredients. Though some may act as medicines, others may act as toxins and bring irreversible loss to some susceptible organs like liver and kidneys. It is also not possible for the common men to isolate the beneficial ingredients (if present) from the harmful ones as it will require maximum sophistication and it will not be economically viable. Hence, toxicological investigation using laboratory animals is a necessary part of promotion of ethno-medicinal components for common men use in traditional ways.

Streptozotocin (STZ, Zanosar) is a naturally occurring chemical produced by the soil microbe *Streptomyces achromogene* that is particularly toxic to the insulin-producing beta-cells of the pancreas in mammals. It is used in medicine for treating certain cancers of the Islets of Langerhans and used in medical research to produce animal models for Type 1 and type 2 diabetes[2, 3, 4 & 5]. Nicotinamide is an amide derivative of nicotinic acid or vitamin B3 which is an active antioxidant. When streptozotocin is administered along with nicotinamide Type 2 diabetes occurs[6].

Present investigation deals with the role of water-extract of *Syzygium cumini* seeds on streptozotocin-nicotinamide-induced type-2 diabetes mellitus of Albino Rat. In this study Glucose Tolerance Test (GTT) was done both in normal control and experimental animals for the comparative evaluation of their serum glycaemic indexes.

GST (Glutathione S-Transferase) and CYP (Cytochrome p450) are two main enzymes responsible for xenobiotic metabolism[7,8]. In case of entrance of any harmful foreign component to the body, the levels of their activities rise several times in various tissues including liver to detoxify the systems. GST and CYP activities in liver samples are investigated both in normal-control and the ethno-medicine administrated animals to find out the probable xenobiotic stress (which may lead to intoxication) on the administration of *S. cumini* seed-extract.

In intoxication free radicals including H^0 & OH^0 are formed which in turn bring lysis of the lipid bi-layer of the cell membrane by oxidative degradation of lipids. It is the process in which free radicals "steal" electrons from the lipids in cell membranes, resulting in cell damage. This process proceeds by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids, because they contain multiple double bonds in between which methylene -CH_2- groups lie, that possess especially reactive hydrogen molecules. This phenomenon is known as “lipid peroxidation (LPO)”. As a result of this, the cells of soft tissues like Liver, Kidney and Gill etc. are destroyed and the contents of the cell are released to the body fluid (serum).[9] The activity of the enzymes AST (Aspartate Transferase), ALT (Alanine Transferase) and ALP (Alkaline phosphatase) are mainly localized in the hepatocytes. Hepatocyte destruction increases their level of activity in blood serum.

Investigations of Aspartate Transferase (AST), Alanine Transferase (ALT) and Alkaline Phosphatase (ALP) activities in blood serum of the study animals are done along with study of Liver Lipid Peroxide (LLPO) to evaluate probable hepatotoxicity on administration of the ethno-medicine.

Study on the levels of Urea, Creatinine and Uric acid levels in blood serum were also done in normal-control and experimental animal groups to find out probable nephrotoxicity.

MATERIALS AND METHODS

The ripe fruits of *S. cumini* are de-pulped and the obtained seeds are sun-dried. These seeds are powdered using mixer- grinder and kept in air tight glass container. 20g of seed-powder is soaked in 1000ml of deionized water overnight and filtered through Whatman’s organic-grade filter paper. Filtrate is vacuum-

dried at 50 ± 2^0 C and powdered, kept in air tight container in deep freeze to use as the ethno-medicinal component. This is used within 3 days of preparation.

Healthy Albino Rats (Wister variety) of either sex are reared fed on pulses, gram and bread. After acclimatization they are divided into four experimental groups (5 animals per group), namely Group-I, Group-II, Group-III and Group-IV. Type-2 diabetes mellitus is induced to each rat of Group-III & IV with administration of streptozotocin dissolving in normal saline, as a single dose of 60 mg/kg body weight 15 minutes after administration of nicotinamide (120mg/kg body weight). Administrations are done intraperitonealy using ophthalmological micro-syringe under light ether anesthesia[6]. 400mg of *S. cumini* seed-extract/kg body weight is administrated orally to Group-II & Group-IV Rats daily for 4 weeks parallely to streptozotocin-nicotinamide administration.

On 29th day the Rats are kept on fasting overnight (18 hours). For glucose tolerance test (GTT) oral administration glucose is done to each of the Rats of the four groups at a dosage of 2 gm/kg body weight. For this test, blood samples are collected at 0, 30, 60, 90 and 120 minutes of glucose administration under light ether anesthesia.

Blood samples are collected retro-orbitally from the inner canthus of the eye using micro-haemorit capillaries under light ether anesthesia and kept in separate labeled micro-centrifuge tubes[10]. These are allowed to clot at room temperature for 20 minutes. The sera of respective blood samples are extracted by centrifugation and kept in separate labeled micro-centrifuge tubes in 0^o to 4^oC. Then animals are sacrificed by high dose of ether anesthetization and livers are dissected out and kept in deep freeze in proper labeled vials.

A proper hygienic condition is provided to the study animals. No juvenile or pregnant individual is applied for the experimental purposes. The standard guidelines prescribed by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA 2003 [11]) are followed during the study.

Measurement of Serum glucose level (for GTT) is done by using Glucose estimation kit (DPSE/GOD-POD).[12] Serum-AST activity is measured by using AST (GOT) reagent kit (IFCC/Kinetic)[13]. Serum-ALT activity is measured by using ALT (GPT) reagent kit (IFCC/Kinetic)[14]. Serum-ALP activity is measured by ALP reagent kit (GSCC/Kinetic).[15] Measurement of Liver Lipid Peroxidation is done by the photometric evaluation of molar extinction co-efficient of thiobarbituric acid[16]. GST activity in liver is measured by using GST Assay kit (Kinetic).[17] CYP activity in liver is measured by using Cytochrome p450 assay kit (Kinetic)[18]. Total protein estimation in liver samples is also done[19] and GST and CYP activities are expressed as activity per mg of liver protein. Serum Urea level is measured by using Urea reagent kit (Mod. Berthelot)[20]. Serum Creatinine level is measured by using Creatinine reagent kit (Alkaline Picrate).[21] Serum Uric Acid level is measured by using Uric Acid reagent kit (Uricase/PAP).[22]

Glucose, AST, ALT, ALP and Creatinine assay kits are procured from Ranbaxy-RFCL (India) LTD. Urea and Uric acid assay kits are procured from Crest Biosystems (India) LTD. GST and CYP assay kits are procured from Sigma-Aldrich Inc. (USA). Folin-Ciocalteu's phenol reagent (used in protein estimation) is procured from Sigma-Aldrich Inc. (USA). Thiobarbituric acid is procured from Research Fine Chem (India) LTD. Streptozotocin and Nicotinamide are procured from Sigma-Aldrich Inc. (USA). The other regents and chemicals are procured from Ranbaxy-Ranchem, (India) LTD.

All the biochemical investigations and evaluations are done in a semi automated biochemistry analyzer ("Lab Life Chem-Master" manufactured by Ranbaxy- Diagnova LTD) with proper programming.

RESULTS

Results obtained during the period of investigation are statistically analyzed[23] with the help of MS Excel and presented in the following tables-

Table 1: Showing serum glucose contents of different experimental Rat Groups during Glucose Tolerance Test

Experimental Rat Groups. → / Time of blood sample collection (in Minutes) ↓	Group-I Normal Control Rats (mg/dl)	Group-II Rats fed with. *S. cumini* seed-extract (mg/dl)	Group-III Streptozotocin-Nicotinamide injected (Diabetic) Rats (mg/dl)	Group-IV Streptozotocin-Nicotinamide injected (Diabetic) Rats fed with *S. cumini* seed-extract (mg/dl)
0	76.48±0.124	72.26±0.108 -5.52% *	99.34±0.103 +29.89% *	85.38±0.058 -14.05% *
30	122.38±0.102	117.30±0.114 -4.15% *	163.56±0.108 +33.65% *	131.22±0.139 -19.77% *
60	114.44±0.150	109.24±0.108 -4.54% *	161.52±0.153 +41.14% *	122.32±0.196 -24.27% *
90	102.30±0.073	97.30±0.118 -4.88% *	156.34±0.051 +52.83% *	109.26±0.103 -30.11% *
120	87.42±0.102	82.26±0.150 -5.90% *	149.32±0.097 +70.81% *	93.56±0.123 -37.34% *
"*" indicates Significant at p<0.001 "+…%" and "-…%" indicate percent increase and percent decrease respectively.				

Table 2: Showing different parameters of hepatic and renal functions of experimental Rat Groups on administration of Streptozotocin-Nicotinamide and *S. cumini* seed-extract

Experimental Rat Groups. → / Study parameters ↓	Group-I Normal Control Rats	Group-II Rats fed with *S. cumini* seed-extract	Group-III Streptozotocin-Nicotinamide injected (Diabetic) Rats	Group-IV Streptozotocin-Nicotinamide injected (Diabetic) Rats fed with *S. cumini* seed-extract
Serum AST (IU/l)	274.290±0.012	261.53±0.078 -4.65% *	292.41±0.091 +6.61% *	277.55±0.106 -5.08% *
Serum ALT (IU/l)	73.190±0.032	71.53±0.089 -2.54% *	78.49±0.110 +7.24% *	74.542±0.141 -5.03% *
Serum ALP (IU/l)	814.556±0.115	798.47±0.127 -1.97% *	842.638±0.107 +3.45% *	835.466±0.079 -0.85% *
Liver Lipid Peroxide (n mol/mg)	233.882±1.16427	227.28±0.811622 -2.82279 *	277.782±1.961674 +18.77015 *	261.036±1.148819 -6.02847 *
Liver GST (µmol/min/mg of protein)	0.3516±0.006423	0.3008±0.001393 -14.4482 *	0.3866±0.002694 +9.954494 *	0.365±0.001761 -5.58717 *
Liver CYP (µmol/min/mg of protein)	370.908±0.979339	347.936±1.783989 -6.19345 *	399.85±4.554312 +7.803013 *	382.89±1.129889 -4.24159 *
Serum Urea (mg/dl)	16.438±0.014	16.446±0.011 +0.049% N.S.	21.552±0.013 +31.11% *	18.040±0.034 -16.29% *
Serum Creatinine (mg/dl)	1.234±0.007	1.246±0.012 +0.972% N.S.	3.54±0.011 +186.87% *	1.93±0.014 -45.48% *
Serum Uric Acid (mg/dl)	1.352±0.013	1.348±0.012 -0.296% N.S.	3.922±0.009 +190.09% *	2.070±0.023 -47.19% *
"N.S." indicates Not significant and "*" indicates Significant at p<0.001 "+…%" and "-…%" indicate percent increase and percent decrease respectively.				

DISCUSSION

It has been found that the oral administration of the seed extract of *S. cumini* lower the serum glucose level both in non diabetic (Group-II) and Streptozotocin-Nicotimide induced diabetic (Group-IV) individuals.

In Normal Control (Group-I) Rats the serum glucose level after overnight fasting is found to be 76.48±0.124. After oral administration of glucose (2gm/kg) it becomes 122.38±0.102 mg/dl after 30 minutes and 144.44±0.150 mg/dl after 60 minutes respectively. It decreases to 102.30±0.073 mg/dl and 87.42±0.102 mg/dl on 90^{th} and 120^{th} minutes respectively.

Similar trend is observed in Rats fed with *S. cumini* seed-extract (Group-II). It increases from 72.26±0.108 mg/dl to 117.30±0.114 mg/dl on 30^{th} minute and 109.24±0.108 mg/dl on 60^{th} minute after oral glucose administration which gradually decreases to 97.30±0.118 mg/dl and 82.26±0.150 mg/dl on 90^{th} and 120^{th} minutes respectively. In this group significant decrease ($p<0.001$) of serum glucose levels is observed with -5.52%, -4.15%, -4.54%, -4.88% and -5.90% deviations respectively on 0, 30, 60, 90 and120 minutes of glucose administration from Normal Control Rats.

On induction of Type-2 diabetes with Streptozotocin-Nicotinamide (Group-III Rats) the serum glucose levels raise significantly ($p<0.001$) up to 99.34±0.103 mg/dl, 163.56±0.108 mg/dl, 161.52±0.153 mg/dl, 156.34±0.051 mg/dl and 149.32±0.097 mg/dl on 0, 30, 60, 90 and 120 minutes respectively on oral glucose administration with +29.89%, +33.65%, +41.14%, +52.83% and +70.81% deviations respectively from Normal Control Rats.

Significant decrease ($p<0.001$) of serum glucose levels are marked on oral administration of *S. cumini* seed-extract to diabetic Rats (Group-IV) from diabetic Rats of Group-III which are not administered with the ethno-medicinal component. Here, serum glucose levels are 85.38±0.058 mg/dl, 131.22±0.139 mg/dl, 122.32±0.196 mg/dl, 109.26±0.103 mg/dl and 93.56±0.123 mg/dl on 0, 30, 60, 90 and 120 minutes respectively from glucose administration with -14.05%, -19.77%, -24.27%, -30.11% and -37.34% deviations respectively from Group-III Rats.

In hepatic function tests it is observed that serum AST activity decreases significantly ($p<0.001$) with from 274.29±0.012 IU/l to 261±53 IU/l in Group-II Rats from Normal Control Rats with a deviation of -4.65%. Similar trend of significant decrease is marked with serum ALT, serum ALP and LLPO with -2.54%, -1.97% and -2.82279 respectively. These indicate that this ethno-medicinal component has no hepatototoxic property, but it has hepatostimulant and hepatopotective properties. Significant ($p<0.001$) rise of AST, ALT and ALP activities and LLPO level are observed in Group-III (Diabetic) Rats with deviations of +6.61%, +7.24%,+3.45%and +18.77015 respectively, which is due to gluconeogenic destruction of hepatocytes. But the activities of these enzymes are significantly ($p<0.001$) decrease with deviations of -5.08%, -5.03%, -0.85% and -6.02847
respectively when diabetic rats (Group-IV) are administered with the ethno-medicine. It is due to the rejuvenetive role of the ethno-medicine to hepatic tissue.

In renal function tests no significant augmentation of the levels of Urea, Creatinine and Uric Acid in blood are observed in the Rats fed with this ethno-medicine(Group-II) from normal control (Group-I) Rats. This indicates that this ethno-medicine has no nephrotxic property. Significant ($p<0.001$) rise of the mentioned parameters are observed in Streptozotocin induced diabetic (Group-III) Rats with 31.11%, 186.87% and 190.09% deviations respectively from Normal Control Rats. It is nothing but due to hyperglycaemic stress on nephrons causing renal dysfunction. The levels of Urea, Creatinine and Uric Acid in blood are observed to decrease in the diabetic Rats fed with this ethno-medicine (Group-IV) with deviations of -16.29%, -45.48% and -47.19% respectively from Rats of Group-III. This is due to the decrease of hyperglycaemic stress on the nephrons.

Studies on GST and CYP in liver samples revealed that, the levels of their activities slightly decreased ($p<0.001$) in group-II rats from normal control on administration of *S. cumini* seed-extract. Similar picture is observed in case of diabetic and ethno-medicine administered diabetic rat groups (group-II and IV). It indicates that the ethno-medicine do not create xenobiotic stress on administration rather it has some detoxifying property.

CONCLUSION

From this study it can be concluded that water-extract of *S. cumini* seeds definitely have some revital properties which can reduce destruction of pancreatic β-cells and to enhance insulin secretion by them. This property may be helpful in management of Type-2 diabetes mellitus.

Study of AST (Aspartate Transferase), ALT (Alanine Transferase), ALP (Alkaline Phosphatase) activities in blood samples of the experimental animals revealed that no hepatotoxicity occurs from the use of *S. cumini* seed-extract. Rather, it plays a hepatostimulant and hepatoprotective role.

Investigation of Urea, Creatinine and Uric Acid levels in blood samples of the study animals lead to a conclusion that no nephrotoxicity occurs from the use of this ethno-medicinal component in traditional way; it reduces levels of these parameters in blood of diabetic Rats significantly due to the reduction of hyperglycaemic stress on kidneys.

Study of the activities of GST and CYP in liver samples of the study animals indicated that no major toxicity occurs for the administration of this ethno-medicine.

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