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ANTI-DIABETIC, HYPOLIPIDEMIC AND HEPATO-RENAL PROTECTIVE EFFECT OF A NOVEL FERMENTED BEVERAGE FROM *SYZYGIUM CUMINI* STEM

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Keywords:

Syzygium cumini; Jamun; diabetes; lipid profile; hepatic and renal function; wine

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ABSTRACT: In view of therapeutic values associated with *Syzygium cumini* and increasing consumer demand for functional foods, a non-conventional alcoholic beverage prepared from the stem of *Syzygium cumini* was evaluated for its efficacy against streptozotocin-induced diabetes mellitus in male wistar rats. The beverage was observed to be safe for consumption as it did not produce any anomalies in the biochemical markers of liver and kidney of normal rats, rather exerted protective effects on hepatic and renal functions by improving the levels of biochemical indicators. After an oral administration of a daily single of dose of 4ml/kg for a period of 30 days, stem beverage was able to rectify the abnormalities in blood glucose levels, lipid profile and atherogenic index of diabetic rats. The beverage also arrested the deviations in hepatic and renal functions of diabetic rats. Histological studies of pancreas also confirmed the anti-diabetic behaviour of stem beverage. The pharmacological properties of the beverage can be ascribed to the presence of various phenolics and tannins in it. The use of this beverage for the management of diabetes mellitus and associated dyslipidemia is suggested.

INTRODUCTION: Diabetes mellitus (DM) is a severe metabolic disorder involving many structural and functional complications. It is commonly associated with dyslipidemia, increased levels of reactive oxygen species and its prolonged occurrence may induce retinopathy, neuropathy, nephropathy and other serious complications. [1] Light to moderate consumption of red wine has been related to improved blood glucose control, redox status along with ameliorated dyslipidemia, inflammation and cardiovascular protection, both in animals and human trials.[2]

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This potency of red wine has been ascribed to the presence numerous bioactive compounds in red grapes as well as to the presence of ethanol in the drink. Studies are also being focused upon other plant based functional beverages that impart therapeutic benefits in medical conditions. [3] In this context, *Syzygium cumini* (also known as Jamun in Hindi) is a potential tree with well-established anti-diabetic efficacy. The ameliorative effects of *S. cumini* stem on diabetes are already reported in the literature.

Intervention with bark extract has been found to increase the levels of insulin and C-peptide in streptozotocin induced diabetic rats. [4, 5] The anti-diabetic potential and other biological efficacies of the stem are attributed to due to the presence of compounds like β-sitosterol, kaempferol, gallic acid, ellagic acid etc. in it. [6]

Further, it is a well-known fact that polyphenols and/or other bioactive compounds are extracted better with ethanol or ethanol-water as a solvent and in the biological system also, ethanol improves the bioavailability of polyphenols. [7, 8] Therefore, this study was aimed at producing a fermented alcoholic beverage from *S. cumini* stem, as the constituency of the beverage was believed to be a better extraction system for pharmaceutical compounds. The next step involved the evaluation of the use of this drink as a functional beverage therapy against DM in animal model as well as comparison with its extract, a commercial red wine and a standard drug (glibenclamide), as discussed at length.

MATERIALS AND METHODS:

Microorganism: *Saccharomyces cerevisiae* MTCC 786 used in the study was procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India.

Drugs and reagents: Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St. Louis, USA). Glucose-oxidase peroxidase glucometer (Abbott India Ltd.) and Glibenclamide (Daonil, Emcure Pharmaceuticals India Ltd.) were procured from a local chemist. Red wine (Tocornal Merlot 2011) was received as a gift from Mr. Gaurav Kumar.

Ethics statement: The experiment protocols were approved by the Institutional Animal Ethics Committee (approval ID: PU/IAEC/F/15/84, dated 15/9/2015) and performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, on animal experimentation. All efforts were made to minimize the suffering of animals.

Animals: Male wistar rats (200–250 g) were procured from Central Animal House, Panjab University, Chandigarh (India). The animals were housed under standard laboratory conditions, maintained on a 12:12 h light:dark cycle and had free access to standard pellet diet (Ashirwad Industries Pvt Ltd, Punjab, India) and water *ad-libitum*.

Plant material and extract preparation: *Syzygium cumini* stem were collected from Panjab University campus, Chandigarh, India in 2015. The plant specimen was identified by Prof. Promila Pathak, Department of Botany, P.U., Chandigarh and a voucher number 21045 was assigned to it. A specimen of the plant was deposited in the herbarium of Department of Botany, PU, Chandigarh. For preparation of extract, Jamun leaves were separated from stem. The stems were cut into small pieces and added to 500 mL distilled water at the rate of 15% (w/v) in a 1L Erlenemeyer flask. The mixture was then boiled for 20 min and the unfiltered extract was used for further experiments.

Beverage production: Five hundred mL of the unfiltered Jamun stem extract was taken in 1L amber colored Borosil glass reagent bottle and its total soluble solids (TSS) was adjusted to 20˚ Brix using cane sugar and pH was kept at 4.5 using citric acid. This was followed by the supplementation of diammonium hydrogen phosphate and magnesium sulphate @ 0.1% (w/v) each, potassium metabisulphite @ 100 ppm and 10 % (v/v) inoculum having 1×10^8 viable cells of *Saccharomyces cerevisiae*. Fermentation was carried out for 20 days in stationary state at 22±2˚C in a BOD incubator. After completion of fermentation, beverage was filtered. Repeated racking and siphoning was done after a period of 7 days for 3 weeks, after which the beverage was stored in amber colored glass reagent bottle for further use.

Analysis of beverage: Bio-chemical analysis of beverage was carried out by standard protocols. The parameters studied include ethanol content [9], titratable acidity (AOAC), total phenolic content [10], total antioxidant capacity [11].

Induction of diabetes in rats: Before inducing diabetes, rats were fasted for at least 16 h, after which a single dose of 55 mg/kg body weight (bw) STZ, prepared in citrate buffer (pH 4.5, 0.1 M) was injected into the rats intraperitoneally (i.p.). Another group of rats serving as a control was injected with citrate buffer only. Establishment of diabetes was confirmed after 48 h of STZ injection by estimating plasma glucose levels using enzymatic glucose oxidase-peroxidase (GOD-PAP) diagnostic kit method after collecting blood samples through tail vein.

The rats displaying fasting plasma glucose levels higher than 250 mg/dl [12] were selected and used in the study.

Experimental design: Rats were randomly distributed into ten groups of six rats each. The study was continued for 4 weeks. Different treatment groups were as follows:

Group I- Control rats which received isotonic saline per os (p.o.) at a daily dose of 4mL/ kg bw/day for 4 weeks after injecting citrate buffer (i.p.) on 1st day.

Group II- Beverage *per se* group in which Jamun beverage was orally administered with gavage to non-diabetic rats at a daily dose of 4mL/ kg bw/day (i.e. 280ml/70kg body weight, per day).

Group III- Extract *per se* group in which stem extract was orally administered with gavage to non-diabetic rats at a daily dose of 4mL/ kg bw/day.

Group IV- Diabetic control in which animals were injected with STZ and administered isotonic saline (p.o.) daily at a dose of 4mL/ kg bw/day.

Group V- Jamun beverage prophylactic group in which animals received Jamun beverage daily (p.o.) for a period of 15 days followed by induction of diabetes and subsequent administration of beverage again for 15 days at a dose of 4mL/ kg bw/day.

Group VI- Jamun extract prophylactic group rats received the Jamun extract (p.o.) daily at a dose of 4mL/ kg bw/day for a period of 15 days, followed by induction of diabetes and again administered with extract for a period of 15 days post diabetes induction.

Group VII- Jamun beverage therapeutic group in which animals were injected with STZ and then were fed with Jamun beverage (p.o.) 4mL/ kg bw/day for 4 weeks.

Group VIII-Jamun extract therapeutic group in which animals were injected with STZ and then were fed with Jamun extract (p.o.) 4mL/ kg bw/day for 4 weeks.

Group IX- Red wine therapeutic group, rats were injected with STZ and then were fed with red wine 4mL/ kg bw/day for 4 weeks.

Group X- Glibenclamide treated group, in which rats were first diabeticized and were then orally administered with Glibenclamide at a daily single dose of 0.5mg/kg bw/day for 4 weeks.

After 4 weeks, animals were sacrificed. Blood was collected for biochemical analysis and pancreas tissues were fixed in 10% formalin solution for histological studies. Protocols used in the study were approved by the Institutional Ethics Committee and were in accordance with the Guidelines for the Humane Use and Care of Laboratory Animals (INSA 2000).

Estimation of Fasting Blood Glucose and Insulin levels: Plasma glucose levels were estimated on weekly basis by enzymatic glucose oxidase-peroxidase diagnostic kit (Free style Optima Plus, Abbott India Pvt. Ltd.) after collecting blood samples through tail vein of the animals. Serum insulin levels of the rats were estimated with rat insulin ELISA kit from Crystal Chem Inc. (US).

Histopathological studies: A portion of pancreas were fixed in neutral buffered formalin solution (10%) and then embedded in paraffin. 5µm thick sections of the respective samples were cut, deparaffinized and stained with hematoxylin and eosin. Thin sections were made into permanent slides and examined under transmitted light microscope.

Lipid profile analysis: Lipid profile analysis was done in serum to estimate the total cholesterol (TC), triglycerides (TG) and high-density lipoprotein cholesterol (HDL-C) using colorimetric kits obtained from Reckon India Pvt. Ltd. Low-density lipoprotein-cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C) and Atherogenic Index (AI) were calculated.[13-15]

Hepatic and renal function analysis: Hepatic and renal function analysis were done by the estimating the levels alanine transaminase (ALT), aspartate transaminase (AST), alkaline Phosphatase (ALP), creatinine, blood urea nitrogen (BUN), and uric acid in serum with the help of commercially

available kits obtained from Reckon India Pvt. Ltd and Accurex Biomedical Pvt. Ltd., India.

Statistical analysis: All data are expressed as the mean ± SD of six animals per group. Statistical differences between the groups were analysed with SPSS software, version 11.0. Two-way analysis of variance (ANOVA) was performed for Blood Glucose levels and one-way analysis of variance (ANOVA) for lipid profile, hepatic and renal functions, followed by multiple comparisons using Holm-Sidak method. The level of significance was set at $p<0.05$.

RESULTS: Over the years, the demand for functional nutrition has increased and this has blurred the distinction between pharma and nutrition. Advanced research studies have shown that certain foods may exert beneficial roles in some diseases apart from imparting basic nutrition.[16] Keeping this in view, the focus of the study was to assess the safety and pharmacological effect functional beverage prepared from *S. cumini* stem on diabetic animals.

Bio-chemical structure of beverage: A clear light-brown colored beverage was obtained from the fermentation of Jamun stem. The wine was composed of an ethanol content of 8.0±0.03% v/v, a pH of 3.4±0.02, TSS of 2˚Brix, titratable acidity of 0.50±0.01gTartaric acid/100mL and reducing sugars were 0.44±0.02 g/100mL. Previous studies have reported the production of an alcoholic beverage from fruit of *S. cumini* and has shown an ethanol content of 6-8.82% (v/v), a pH of 3.25-4.5 and acidity of 0.61-1.1gTartaric acid/100 mL. [17-19] These observations show that our beverage is similar to those of *S. cumini* fruit wines in terms of its physico-chemical attributes. In our study, total anti-oxidative capacity of the beverage was found to be 27.09±0.1 µM/mL whereas the total phenolic content (TPC) was 331.5±0.15µg/mL which is in agreement with previous studies that have shown TPC content of *S. cumini* stem. [20, 21]

Anti-diabetic efficacy of beverage: In animal studies, STZ injection led to induction of diabetes and a typical case of hyperglycemia was observed. On third day after injection, FBG detected in diabetic rats was 315±27.4 mg/dL, which rose to 455±37.67 mg/dL at 30th day of the study.

In contrast to this, control animals exhibited an FBG of 81±6 mg/dL throughout the course of the study. Beverage administration to non-diabetic rats did not produce any negative effect as the rats continued to display normal FBG levels, **Fig. 1a**.

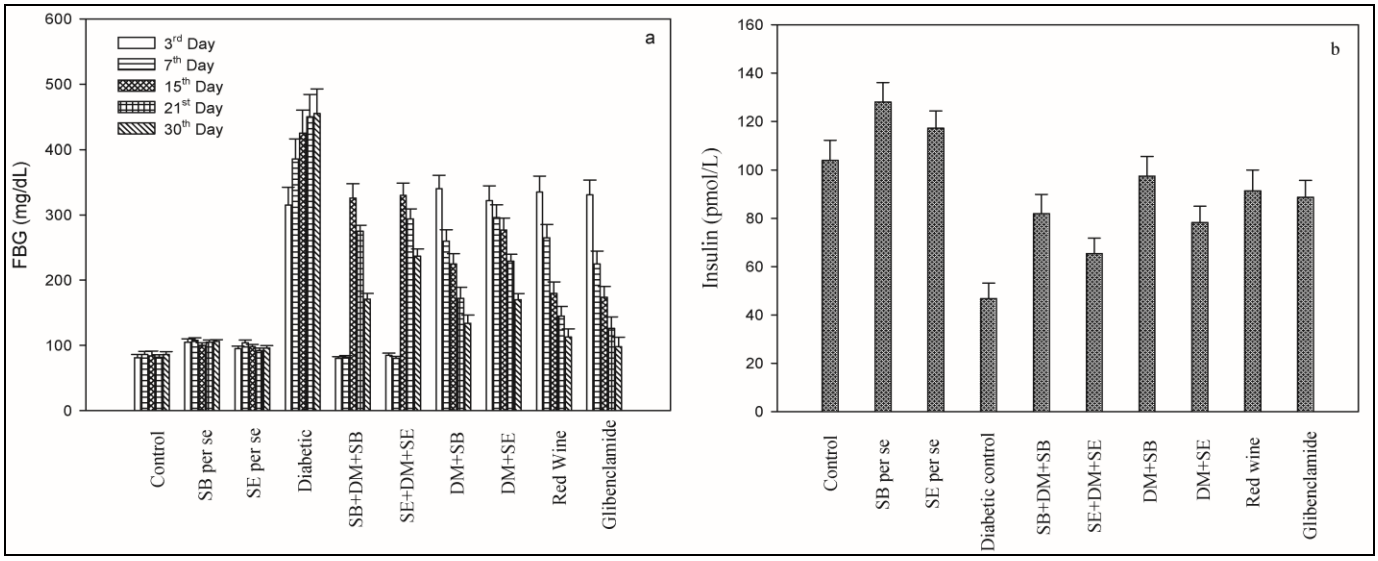

FIG. 1: EFFECT OF STEM BEVERAGE ADMINISTRATION ON FASTING BLOOD GLUCOSE LEVELS (FBG) (A) AND SERUM INSULIN LEVELS (B) OF DIABETIC RATS. SB+DM+SB DENOTE BEVERAGE PROPHYLACTIC TREATMENT AND DM+SB DENOTE THERAPEUTIC TREATMENT WITH STEM BEVERAGE, RESPECTIVELY. SE+DM+SE DENOTE EXTRACT PROPHYLACTIC TREATMENT AND DM+SE DENOTE THERAPEUTIC TREATMENT WITH STEM EXTRACT, RESPECTIVELY. FOR EACH DIETARY TREATMENT, AFTER THE ESTABLISHMENT OF DISEASE MEANS OF CONTROL, BEVERAGE *PER SE*, SB+DM+SB AND DM+SB DIFFER FROM DIABETIC GROUP SIGNIFICANTLY WITH P < 0.01.

The effect of beverage on the diabetic rats was found to be significant. During the prophylactic treatment, after the establishment of the disease, beverage was able to lower down FBG levels and brought them from 326±21.8 to 171±8.6 mg/dL. More pronounced effect of the beverage was observed in therapeutic group in which, after administration of the beverage dose for 30 days, FBG levels were found to be in near-normal range.

From 340±20.8 mg/dL on the third day of STZ injection, during the study period FBG levels steeped down to 260±17.3, 225±15.4, 190±17 mg/dL on 7^{th}, 14^{th}, 21^{st} day and subsequently 134±12.5 mg/dL on 30^{th} day. Overall, a 70% reduction in FBG levels was observed on the 30^{th} day of therapeutic treatment. Extract treatment also lowered FBG, but showed lower efficacy as compared to the beverage as the observed FBG was 174±10.2 mg/dL on 30^{th} day (**Fig. 1a**). Histological studies of rat pancreas also confirmed the curative activity of beverage. It is evident from the photomicrographs that beverage administration restored the impaired the damaged and diminished islets caused by STZ injection morphologically also, as depicted in **Fig. 2**. In contrast to this, untreated rat pancreas showed absence of islets. Stem beverage however, led to regeneration and repair of islets in diabetic rats. Administration of red wine and glibenclamide also improved the condition of damaged islets. These observations explain the increased insulin levels in various treatment groups.

In comparison to diabetic controls, which exhibited an insulin level of 46.8±6.4 pmol/L, stem beverage therapeutically and prophylactically treated animals possessed an insulin level of 97±8.1pmol/L and 82±7.9pmol/L. Control animals showed 104±8.2 pmol/L serum insulin. As shown in Fig. 1b, treatment with stem extract and red wine also increased serum insulin levels in diabetic animals.

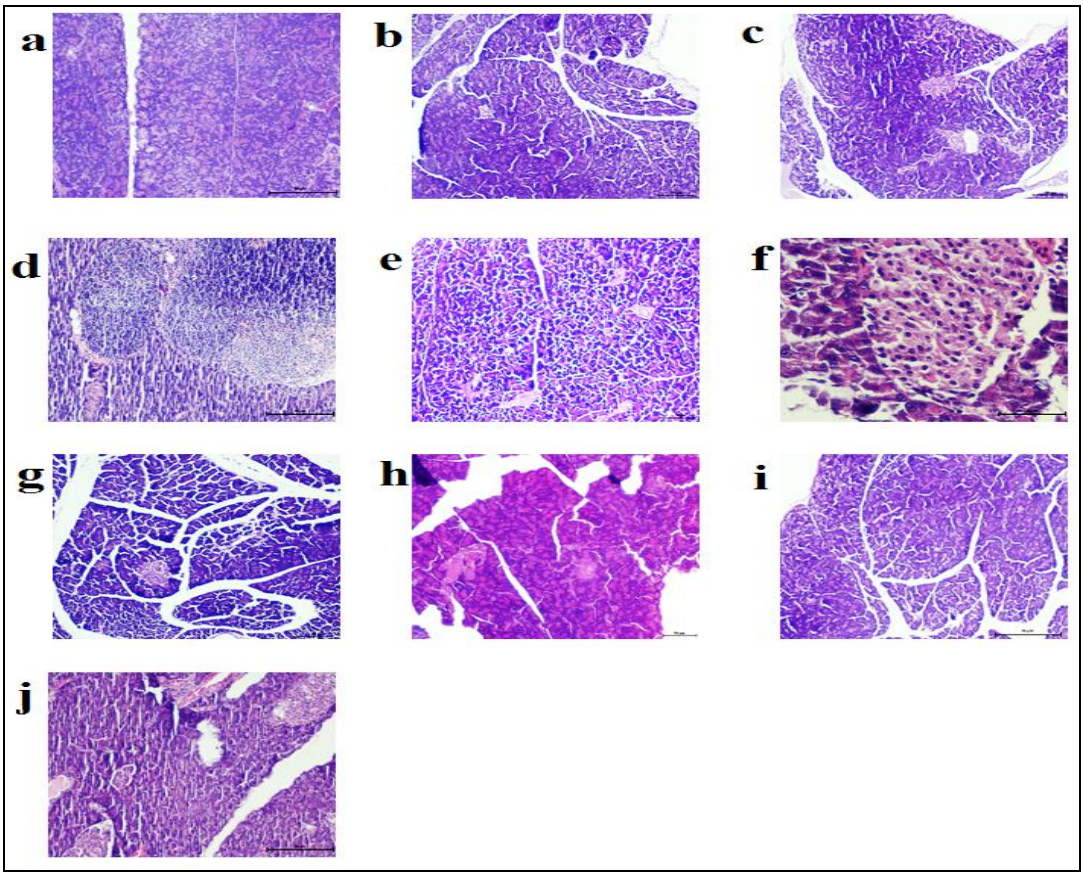

FIG. 2: HEMATOXYLIN AND EOSIN STAINED SECTIONS OF RAT PANCREAS. NORMAL MORPHOLOGY OF PANCREAS OF CONTROL GROUP (a), BEVERAGE AND EXTRACT PER SE GROUP (b, c), NECROTIC PANCREAS OF DIABETIC RAT SHOWING SEVERE INFLAMMATION (d), PANCREAS OF RAT PROPHYLACTICALLY TREATED WITH BEVERAGE AND EXTRACT SHOWING HEALING ISLETS (e, f), NORMAL MORPHOLOGY OF PANCREAS AFTER THERAPEUTIC TREATMENT BY BEVERAGE (G) PANCREAS OF RAT TREATED WITH EXTRACT THERAPEUTICALLY (h), RED WINE TREATED RATS'S PANCREAS SECTION (i), GLIBENCLAMIDE TREATED RAT'S PANCREAS (j). H AND E×100.

Effect of beverage on Lipid profile: The lipid profile of the rats was also found to be significantly affected by stem beverage. Administration of the beverage lowered total cholesterol (TC), LDL-C, atherogenicity (AI) and increased HDL-C in the rats as compared to control rats, thereby exerting cardio-protective effects in animals. Serum cholesterol levels of non-diabetic rats fed with beverage (*per se*) was observed to be 50.75±2.6 mg/dL as compared to 69±2.76 mg/dL of the control group. On a contrary, diabetic rats displayed a TC content of105±8.8mg/dL. Administration of beverage brought down the cholesterol to 78.29±5.8 and 58±3.9 mg/dL in prophylactic and therapeutic treatments, respectively (**Table 1**).

Extract administration however, brought TC to 86.81±6.7 and 67.1±4.2 mg/dL after prophylactic and therapeutic treatment. Similarly, elevated serum triglyceride levels were brought down to 154.4±8.6 mg/dL and 129.4±7.4mg/dL in the beverage prophylactically and therapeutically treated animals as compared to 228.11±15.73 mg/dL in untreated diabetic rats. Beverage administration also improved the status of HDL-C from 25.4±1.36mg/dL to 37.5±1.5 mg/dL and 33.4±1.3 mg/dL in therapeutic and prophylactic treatment groups. Clear positive effects were also observed in LDL-C, VLDL-C and atherogenic index (**Table 1**). It was observed that beverage ameliorated the alteration in LDL-C and VLDL-C also as therapeutically treated animals displayed.

TABLE 1: EFFECT OF STEM BEVERAGE ON LIPID PROFILE OF DIABETIC RATS

Animal group	TC (mg/dL)	LDL (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	VLDL (mg/dL)	AI
Control	69±2.76*	41.54±4.6*	108±3.5*	38.81±1.2*	21.6±1*	0.44±0.02*
SB Per se	50.75±2.6*	20.83±3.7*	100±3.9*	40.09±1.5*	20±1.2*	0.39±0.01*
SE Per se	55±2.49*	26.8±4*	106.4±3.7*	41.1±1.18*	21.28±1.14*	0.41±0.01*
Diabetic control	105±8.82	147.19±6.0	228.1±15.73	25.4±1.9	45.62±1.61	0.95±0.064
SB+DM+SB	78.29±5.8*	78.68±3.6*	154.4±8.6*	33.4±1.3*	30.88±1.6*	0.66±0.037*
SE+DM+SE	86.81±6.7*	105.6±5.1*	182.3±9*	27.8±1.4#	36.46±1.8*	0.81±0.057*
SB+DM	58±3.9*	44.75±5.8*	129.4±7.4*	37.5±1.5*	25.88±2.1*	0.53±0.05*
SE+DM	67.1±4.2*	76.63±4.9*	159.2±6.8*	28.1±1.47#	31.84±1.94*	0.75±0.061*
Red wine	74±4.50*	69.89±2.1*	148±7.84*	35.4±1.0*	29.6±1.4*	0.62±0.05*
Glibenclamide	70±4.81*	72.18±4.7*	152±8.1*	31.5±1.4*	30.4±1.36*	0.68±0.061*

SB+DM+SB denote beverage prophylactic treatment and DM+SB denote therapeutic treatment with stem beverage, respectively. SE+DM+SE denote extract prophylactic treatment and DM+SE denote therapeutic treatment with stem extract, respectively. For each dietary treatment, after the establishment of disease, means of Control, Beverage *per* se, SB+DM+SB and DM+SB differs from Diabetic group significantly. *, $p<0.01$ versus Diabetic control. #, $p<0.05$ versus Diabetic control

44.75±5.8 mg/dL LDL-C and 25.88±2.1 mg/dL VLDL-C as compared to 147.04±6.0 and 45.6±1.61 mg/dL in untreated diabetic subjects. Atherogenic index (AI), a predictor of cardiovascular complication was found to be higher in diabetic animals (0.95±0.06) as compared to control animals (0.45±0.002). Out of all the treatment groups, therapeutic treatment by beverage lowered AI most efficiently (AI=0.53±0.05) followed by red wine (0.68±0.061) and glibencamide (0.62±0.054) (**Table 1**). These observations are in agreement with earlier reports which have shown the positive effect of consumption of polyphenol rich foods and beverages on dyslipidemia.

Effect of beverage on Hepato-renal function: The stem beverage was found to be safe for consumption, as no harmful alterations were observations were observed in the hepatic and renal function of the *per se* animals (**Table 2**).

Not only cardiovascular protective, *S. cumini* stem beverage also showed hepato and renal protective effects on diabetic rats. The status of liver and kidney function markers of beverage treated group was found to be better as compared to untreated diabetic subjects. The alterations in the levels of ALT, AST and ALP were found to be controlled by beverage therapy, as shown in **Table 2.** Similarly the markers of kidney function were also influenced by the treatments progressively. Beverage administration led to elimination of excess uric acid and creatinine in diabetic rats. It is evident from the biochemical parameters that the beverage protected liver and kidney of the rats from the abnormalities caused by DM.

The histological images of the treatments also showed the curative effect of wine on liver and kidney of diabetic rats (data not shown).

TABLE 2: EFFECT OF STEM BEVERAGE ON HEPATIC AND RENAL FUNCTION OF DIABETIC ANIMALS

Animal group	ALP (IU/L)	ALT (IU/L)	AST (IU/L)	Creatinine (mg/dL)	BUN (mg%)	Uric acid (mg%)
Control	100.56±2.32*	13.65±1.4*	30.0±1.05*	0.66±0.05	14.08±1.02*	6.63±0.07*
SB Per se	74.62±2.43*	12.37±0.52*	24.3±0.7*	0.583±0.041	11.96±0.8*	5.2±0.06*
SE Per se	80.24±1.2*	15.8±0.63*	26.8±0.64*	0.59±0.036	11.2±0.53*	5.67±0.053*
Diabetic control	200.0±5.80*	61.88±4.7	70.10±1.89	1.48±0.09	29.37±1.97	21.29±1.05*
SB+DM+SB	124±4.57*	32.37±1.78*	42.8±1.30*	0.69±0.07	21.2±1.26*	9.96±0.67*
SE+DM+SE	142.45±3.72*	40.6±1.5*	61.2±1.6*	0.81±0.063	25.4±0.94*	15.3±0.71*
SB+DM	70±4.54*	24.75±1.3*	38.7±0.97*	0.59±0.06	15±1.3*	5.4±0.59*
SE+DM	92.5±3.63*	30.2±0.9*	45.8±1.05*	0.72±0.081	19.3±0.84*	9.8±0.62*
Red wine	72.41±2.62*	30.14±0.71*	46.21±1.34*	0.61±0.07	17.20±0.94*	7.31±0.71*
Glibenclamide	75.4±1.7*	32.4±0.67*	44.93±1.2*	0.57±0.069	16.04±0.87*	7.04±0.69*

DISCUSSION: The prepared stem beverage was found to be similar to that of the already prepared alcoholic beverage from the fruit of *S. cumini* and was found to be rich in phenolic compounds and anti-oxidant potential. The identification of the bio-active components of the stem beverage by LC-MS showed its immense potential in terms of medicinal functionality and therefore, we evaluated it against diabetic mellitus in a rat model. As shown in **Fig. 1**, administration of the stem beverage had a pronounced ameliorative effect on blood glucose levels of diabetic rats. These observations are in agreement with earlier studies which showed curative effect of stem bark of *S. cumini* on diabetic animals. Saravanan and Leelavinothan [4] also showed the effect of aqueous extract of stem bark of *S. cumini* on diabetic rats. They added stem-bark powder to distilled water at a rate of 33.3% (w/v) and concentrated it to obtain a resoluted semi-solid extract which was 15% of the initial volume of the extract.

This extract with a dose of 300mg/kg was able to cause a 64% reduction in FBG levels after 30 days. As compared to this, our beverage which did not incorporate any concentration oriented step led to 70% reduction in FBG displaying a better efficacy. Another report showed that chloroform, petroleum ether, ethanolic and aqueous extract of stem bark of *S. cumini* caused reduction in FBG levels of diabetic rats. Ethanolic and aqueous extracts were found to be the best out of the four extracts used. 500 mg/kg of these extracts controlled and brought FBG to near-normal range after 21 days of administration. [6] The anti-diabetic activities of stem beverage can be ascribed to the presence of myricetin, quercetin, beta-sitosterol, gallic acid, cyanidin, delphinidin, petunidin and malvidin glycosides, catechin, quercetin, and lupeol etc. in the beverage. In our previous report, we confirmed the presence of these bio-actives in the fermented *S. cumini* stem beverage by LC-MS analysis. [22] These compounds impart anti-diabetic activities via various pathways including improving insulin sensitivity, increasing release of insulin from beta cells, insulin-analogy, [23] beta cell regeneration, [24] and amelioration of insulin sensitivity [25] which might have been the mode of action of our beverage also. Anthocyanins, chiefly cyanidin-3-glucoside has been shown to employ insulin-like activity by activating PPARɣ, which is involved in glucose and lipid metabolism as well as glucose and fatty acid uptake. [26]

It was also observed that stem beverage administration improved the level of insulin in diabetic rats and aided in regeneration of islets cells. This must have led to arrest of hyperglycemia in diabetic animals (**Fig. 1).** The effect was also observed in the animals fed with extract, glibenclamide and red wine. Previously, human trials have already shown that the consumption of red wine increases insulin levels [27] and *S. cumini* has been found to regenerate insulin producing cells.

Studies have shown that different extracts of *S.cumini* regenerated insulin producing cells and produced an insulin-analogous effect in addition to glucose stimulated insulin secretion, suggesting improvements in both insulin secretion as well as insulin sensitivity. [28, 29]

Our results are in complete agreement with these findings in terms of improvement in insulin levels and regeneration of pancreatic cells. However, the interesting observation in the present study is the improved efficacy of the fermented alcoholic beverage over its extract and at-par with the standard drug. This shows that fermentation led to enhancement of the pharmacological efficacy of beverage and made it a potent candidate for its use as a nutraceutical food therapy approach.

A typical diet rich in fruits, vegetables and plant beverages has been estimated to provide about 1 g of polyphenols/day and herbal products have been identified to be the richest source of these compounds. Foods and beverages like green tea, cocoa, soy protein, berries, cranberry and raspberry juice etc. have been found to affect blood lipids positively and it has been found that the polyphenols present in them exert their effect by inhibiting critical steps in the intestinal absorption of dietary fat, cholesterol and other lipids, decreasing oxidation of LDL-C and arresting vascular inflammation. [30, 31] In addition to this, alcoholic beverages like red wine have been found to correct the dyslipidemia in diabetic animals and humans. However, the effect was not observed in case of white wine and gin as they are devoid of such bio-active compounds. [32]

The hypolipidemic activity of our beverage may also be due to the action of various tannins comprising proanthocyanins (PA) and flavanols like cyanidin, protocatechuic acid, quercetin which in numerous studies have been found to control cholesterol levels, activate endothelial nitric oxide synthase for prevention of platelet aggregation and adhesion, inhibit LDL-oxidation and possess a regulatory effect on heart diseases. [26, 33] Querectin has also been found to alleviate triglyceride levels in clinical trials. [34] The increase in HDL-C may be due to the presence of ethanol in the beverage. Ethanolic beverages have been found to increase HDL-C and the effect is more pronounced when the beverage is polyphenol-rich. [35, 36]

The hypolipidemic activity of our beverage may also be due to the action of various tannins comprising proanthocyanins (PA) and flavanols like cyanidin, protocatechuic acid, quercetin which in numerous studies have been found to control cholesterol levels, activate endothelial nitric oxide synthase for prevention of platelet aggregation and adhesion, inhibit LDL-oxidation and possess a regulatory effect on heart diseases. [26, 33] Querectin has also been found to alleviate triglyceride levels in clinical trials.[34] The increase in HDL-C may be due to the presence of ethanol in the beverage. Ethanolic beverages have been found to increase HDL-C and the effect is more pronounced when the beverage is polyphenol-rich. [35, 36]

Similar observations were witnessed with the treatments with the extract and red wine. Previous studies have reported the hepato and renal protective effects of *S. cumini* fruit, red wine and polyphenols of Jamun fruit and red wine, respectively. [37-39] It has been found that in mice *S. cumini* pulp extract decreases bile duct ligation induced inflammation and oxidative stress in addition to providing protection against hepatocellular injury. [40] Similarly, significant reductions in liver marker enzymes were observed after 14 weeks of fruit extract administration to hyperlipidemic animals and the authors ascribed the presence of flavonoids for the observed liver protective activity. [41]

Previously, plant flavonoids have also been reported to combat and protect hepatic injuries in animal models (Galisteo et al., 2006). Bio-active compounds of *S. cumini* including gallic acid, kaempferol, quercetin, myricetin, other flavonoids and anthocyanins act as natural anti-oxidants and prevent oxidative stress mediated cellular injuries in the animals. [42] The presence polyphenolic compounds and the observed protective and curative effect of stem beverage in the diabetic rats, advocate its potential of safe functional beverage therapy.

These results suggest that *S. cumini* stem beverage possesses therapeutic effect over diabetes and dyslipidemia similar to that of a red wine and standard drug, glibenclamide. The results also confirm that the stem beverage did not produce any abnormality in the liver and kidney of normal rats, rather it improved their functions. Therefore, it can be considered as safe for consumption and used as an added variety of function based food products.

It is important to note that, not only in diabetics, beverage intake improved the lipid profiles of normal rats also. The atherogenic index of beverage fed non-diabetic rats was also found to be lower as compared to control rats. These observations indicate the protective effect of beverage on cardiovascular parameters. Therefore, the intake of this beverage can be suggested to both non-diabetics and diabetics as it exerted both protective and curative effects. Further studies are required to investigate the pathways through which *S. cumini* stem beverage employs its effects. Clinical trials are also required to be undertaken for confirming the pharmaceutical effects of the beverage in humans.

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