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Evaluation of antidiabetic potential of *Syzygium kanarense* (Talbot) Raizada in streptozotocin- nicotinamide induced diabetic rats

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Abstract

Worldwide diabetes is the major killer disease and the antidiabetic drugs which are in use cause many side effects. Traditionally, some of the *Syzygium* spp. are in use for treating diabetes, and many species are being assessed for their antidiabetic property. This study is to assess the antidiabetic effectiveness of the bark of *Syzygium kanarense* (Talbot) Raizada. The antidiabetic efficacy of methanol and water extracts of the leaf (SKLM, SKLW) and the bark (SKBM, SKBW) were evaluated *in vitro* by the α-glucosidase and α-amylase-inhibitory assays. The *in vivo* antidiabetic activity of the bark was assessed by oral glucose tolerance test (OGTT) and streptozotocin- nicotinamide (STZ-NA)-induced non-obese type 2 diabetic rat model. The serum biochemical parameters and histopathology of the pancreas, liver and kidney were evaluated after 21 days of treatment. The total phenolics and flavonoids were quantified in all the extracts. The antioxidant activity was assessed using the DPPH assay. Administration of SKBM and SKBW to STZ-NA-induced diabetic rats at 300 mg/kg and 600 mg/kg orally for 21 days exhibited statistically significant ($P < 0.001$) and dose-related drop in blood sugar levels, serum lipid and hepatorenal parameters. The extract-treated rats showed rejuvenated islets and increased β-cell density in the pancreas, improved liver architecture and glomerular regeneration without fat deposition. Bark extracts showed the strongest α –glucosidase and α –amylase-inhibitory activity in contrast to the leaf extracts. Antioxidants, phytoconstituents and antidiabetic action, as well as protection against free radical damage, were proved to be significantly correlated.

Keywords

Syzygium kanarense; α- glucosidase; acute toxicity; OGTT; lipid profile; histopathology

Introduction

Diabetes mellitus is a metabolic disease characterized by raised blood glucose levels. The World Health Organisation (WHO) reports that globally 422 million are affected by diabetes annually and is the sixth leading cause of mortality. Diabetes-related deaths increased by 125.5 percent between 1990 and 2017, with India leading the 195 nations and territories evaluated (1). Hyperglycemia is caused by a lack of insulin synthesis or action or both. There is a need to discover newer therapeutic approaches to reduce long-term diabetic consequences. Herbal medicines are often preferred to

treat both acute and chronic disorders due to their efficacy, less side effects and affordability. There are a variety of Indian medicinal plants with anti-diabetic characteristics and other beneficial properties, as well as manufactured herbal medications on the market (2).

Syzygium (Myrtaceae) is one of the largest angiosperm genera, with 1200 species ranging from South-East Africa to the Pacific and South-East Asia. India has 102 species of *Syzygium* of which 44 are endemic (3). *Syzygium* spp. has long been used as medicine in many parts of the world, having proven medicinal benefits for a variety of maladies. Ayurveda, Homeopathy and other folk remedies use herbal formulations of different species of *Syzygium* to treat a variety of acute and chronic ailments (4).

Many species of *Syzygium* have been studied for their anti-diabetic properties. The bark of *Syzygium calophyllifolium*, *Syzygium samarangense* and *Syzygium cumini* have traditionally been used as anti-diabetic (5, 6). In Ayurveda, Unani and various folk medicine systems, *Syzygium cumini* is a frequently used antidiabetic plant (7). There is evidence that *Syzygium cumini* mother tincture, which is used in homeopathic diabetes treatment has a remedial effect on carbohydrate and lipid disorders (8). *Syzygium* spp. is rich in essential oils and other secondary metabolites like phenolics, terpenes, flavonoids, anthocyanins, tannins etc. Crude extracts and isolated bioactive components like myricetin, catechin and gallic acid from various *Syzygium* species have a promising anti-diabetic effect (9, 10).

The leaves were examined for essential oil components and 52 compounds were found, with sesquiterpene hydrocarbons being the most prevalent (12). The leaf ethyl acetate extract showed good antidiabetic activity in STZ-induced diabetic rats without any acute oral toxicity (13). The goal of this study is to evaluate the *in vitro* antidiabetic potential and antioxidant activity and to know the preliminary phytochemical components of the methanol and aqueous extracts of leaf and bark. And also, to evaluate the antidiabetic potential of the bark extracts in streptozotocin (STZ) and nicotinamide (NA) induced diabetic rats.

Materials and Methods

Syzygium kanarense (Talbot) Raizada is a critically endangered, recently rediscovered species having a very limited distribution in India's Western Ghats. Trees can grow up to 30 meters tall and have white, smooth bark (11).

Collection of plant materials

In the month of April, the leaves and stem bark were collected from the Gerusoppa region of Uttara Kannada, Karnataka, India (12°48'525''N and 074°55'232''E). The plant material collected was identified by comparing it with the voucher specimen (HSS-5332) at the herbarium of the Department of Applied Botany, Mangalore University, Karnataka. Freshly collected leaves and bark were cleaned, shade-dried, powdered and used further.

Chemicals

Streptozotocin (STZ) and Nicotinamide (NA) were procured from Sigma, India. Glibenclamide (Daonil, 5 mg tablet) was purchased from Emcure Pharmaceuticals Ltd, India. The rest of the chemicals used were purchased from CDH, India; Himedia, India; SRL India and were all of analytical grade.

Preparation of plant extract

The powdered leaf and bark samples were subjected to soxhlation using methanol. Also, the dried powder was soaked in water for 48 hrs in the water bath at 50 °C to obtain aqueous extract. The extracts were dried in a rotary vacuum evaporator, weighed, and stored at 4 °C until further use. Each extract's yield was calculated and represented as a %.

$$\% \text{ of yield} = \text{weight of the extract} / \text{weight of dried sample taken} \times 100$$

Preliminary phytochemical analysis and quantification

Following the established protocols (14) the leaf methanol and water (SKLM, SKLW), bark methanol and water (SKBM, SKBW) extracts were examined for the presence of flavonoids, alkaloids, tannins, glycosides, terpenes, saponins and steroids. Following the Folin-Ciocalteau method (15) the total phenolic concentration was determined and was expressed as gallic acid equivalents (GAE)/ g of extract. The aluminium chloride method (16) was used for flavonoid quantification and was expressed as quercetin equivalents (QE)/ g of extract. All the experiments were performed in triplicates.

Antioxidant potential of plant extracts

Using the DPPH assay (17) the antioxidant potential was assessed. One mL of different aliquots of the extracts were mixed with 3 mL of 0.004% DPPH and the mixture was left to stand for 30 min at 28 °C under dark. At 517 nm, the absorbance was measured spectrophotometrically (Systronics 104) against methanol blank.

In vitro antidiabetic activity

α-Amylase-inhibitory assay

0.5 mL of α- amylase (2.0 U/mL) was mixed with 1 mL of the extracts of varying concentrations and 6 mM of sodium chloride in 0.02 M phosphate buffer (pH 6.8) following 10 min of incubation. 0.5 mL of 1% starch was added and allowed to react for 10 min. The control sample without enzyme was maintained following the same procedure. Finally, 0.5 mL of 1% dinitrosalicylic acid (DNS) was added and maintained for 10 min in a boiling water bath. Thereafter, the test tubes were set to cool and added 5 mL of deionized water. At 540 nm, the absorbance was measured spectrophotometrically (18).

α-glucosidase-inhibitory assay

50 µL of different concentrations of the extracts were incubated with 100 µL of α- glucosidase (1.0 U/mL) for 10 min. After the addition of 50 µL of p-nitrophenyl glucopyranoside (3 mM) the mixture was left to stand for 20 min at 37 °C. 2 mL 0.1 M sodium carbonate was added as a stopping reagent and the absorbance of released p- nitro-

phenol was measured at 410 nm. The extracts, enzyme, substrate and stop reagents were prepared in 0.2 M sodium phosphate buffer (pH 6.8) (19).

Animals

Ethical approval

Following the guidelines of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), the Government of India, SDM Centre for Research in Ayurveda and Allied Sciences, SDM College of Ayurveda Campus, Udupi, Karnataka, India has approved the protocol (Ref.No: SDMCRA/IAEC/MU-09 dated 07/01/2019).

Healthy, adult male albino Wistar rats weighing between 180-220 g were selected for the experiments. Animals were maintained with a standard pellet diet (Sai Durga feed, Bangalore) and water *ad libitum* and were housed in polypropylene cages maintaining a temperature between 22-25 °C, relative humidity between 50-60% and 12-12 hrs. of light-dark cycle.

Acute oral toxicity study

Following the guidelines of the Organisation for Economic Co-operation and Development- 425 (OECD-425), the acute oral toxicity test was carried out. A test dose of 2000 mg/kg was administered orally to the rats as per the 'Principle of the Limit Test' of OECD. Overnight fasted male rats (n=5) were fed with 2000 mg/kg of SKBM and SKBW extracts. The rats were closely monitored for 4 hrs to look for any behavioral, autonomic and neurological reactions. Further, rats were observed every 24 hrs for mortality throughout the entire study period (i.e., 14 days).

Oral glucose tolerance test (OGTT)

Normal male Wistar rats (n=4) were used for this study. The overnight fasted rats were orally administered with 1 dose of glibenclamide (5 mg/kg), 300 mg/kg and 600 mg/kg of SKBM and SKBW respectively. After 30 min, 2000 mg/kg glucose was fed orally and the blood glucose levels were measured at 0, 30, 60, 120, 180 and 240 min to determine glucose tolerance in various groups (20). Blood sample from the tail vein was tested using an Accu-Chek Active glucometer.

Assessment of antihyperglycemic activity

In the overnight fasted animals, diabetes was induced (21). They were administered (i.p) with 110 mg/kg of nicotinamide (NA). 15 min later 60 mg/kg of streptozotocin (STZ) was given by a single intraperitoneal injection. The solvent for STZ was 0.1M citrate buffer (pH 4.5) and normal saline for NA. Elevated blood sugar levels after 72 hrs of induction confirmed hyperglycemia. Animals having a blood glucose level above 300 mg/dL were employed for the study.

The experimental rats were allotted into 7 groups. Each group had 6 animals.

Group I : Normal control rats (Saline 5 mL/ kg)

Group II : Diabetic control rats (60 mg/kg of Streptozotocin + 110 mg/kg of Nicotinamide)

Group III : Diabetic + Glibenclamide (5 mg/kg)

Group IV : Diabetic + SKBM (300 mg/kg)

Group V : Diabetic + SKBM (600 mg/kg)

Group VI : Diabetic + SKBW (300 mg/kg)

Group VII : Diabetic + SKBW (600 mg/kg)

The diabetic rats were administered with the standard drug and bark extracts orally as given in the above dosage for 21 days to assess their anti-hyperglycemic activity. The blood glucose level was recorded on the 5th, 10th, 15th and 21st day with Accu-Chek Active glucometer. The change in body weight of the rats was monitored in a digital weighing balance. Animals were sacrificed by cervical dislocation. Pancreas, liver and kidney tissue samples were washed with normal saline and stored in phosphate buffered saline (pH 7.4) for further studies.

Assessment of biochemical parameters in serum

On the 21st day, the blood samples were collected by a retro-orbital puncture from the anesthetized overnight fasting rats. The total protein (TP), albumin (Alb), urea, creatinine, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL- C), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in the serum were measured using a commercial kit (Agappe diagnostics Ltd, India). The catalase activity was measured using the method given by Sinha (22). Using Friedewald's equation the concentration of very low-density lipoprotein cholesterol (VLDL- C) and low-density lipoprotein cholesterol (LDL- C) were calculated (23).

Histopathology

The liver, pancreas and kidney tissues were fixed with 10% buffered formalin, sliced, embedded in paraffin blocks, sectioned using a microtome (≈5µm) and stained with hematoxylin and eosin. The sections were observed under 40X using a Nikon Optiphot-2 microscope with Nikon Digital Sight (DS-F1), Japan.

Statistical analysis

The data were statistically analysed using GraphPad Prism software (Version *5.03*). One-way ANOVA followed by the Newman-Keuls Multiple Comparison Test were carried out. The results were expressed as Mean ±SD.

Results

Yield of extracts

The bark extracts showed a higher yield compared to the leaf extracts. The yield was highest in SKBM (19.79±0.31%). The % of extract yield is given in Table 1.

Table 1. % of yield in various extracts

Extracts	% of yield
SKLM	7.24±0.18
SKLW	3.57±0.23
SKBM	19.79±0.31
SKBW	11.21±0.52

Values are the mean (n=3) ± Standard deviation. **SKLM**- *Syzygium kanarense* leaf methanol extract**; SKLW**- *Syzygium kanarense* leaf water extract; **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract

Phytochemical analysis and quantification

All the extracts contained flavonoids, tannins, saponins and glycosides. Terpenes were present in all the extracts except in SKBW. The total phenolic content was highest in SKBM (345.81±7.21 mg GAE/g) and lowest in SKLM (85.42±3.29 mg GAE/g) extracts. The SKBW extract had 199.07± 5.92 mg QE/g flavonoid which is higher in comparison with other extracts (Table 2).

Table 2. Total phenolics and flavonoid contents in leaf and bark extracts.

Extracts	Total phenolics (mg GAE/g extract)	Total flavonoids (mg QE/g extract)
SKLM	85.42±3.29d	74.62±1.73c
SKLW	98.07±5.23c	48.66±1.32d
SKBM	345.81±7.21a	155.55± 7.02b
SKBW	239.39±6.01b	199.07± 5.76a

Values are the mean (n=3) ± Standard deviation, a>b>c>d where significance among extracts, p<0.05 in columns. **SKLM**- *Syzygium kanarense* leaf methanol extract; **SKLW**- *Syzygium kanarense* leaf water extract; **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract

Antioxidant potential of plant extracts

All the extracts showed significant DPPH radical scavenging activity (Fig. 1). The half-maximal inhibition of both leaf and bark extracts was compared with the standard ascorbic acid (12.56 ± 0.76 µg/mL). This showed that bark extracts have higher DPPH radical scavenging activity compared to leaf extracts.

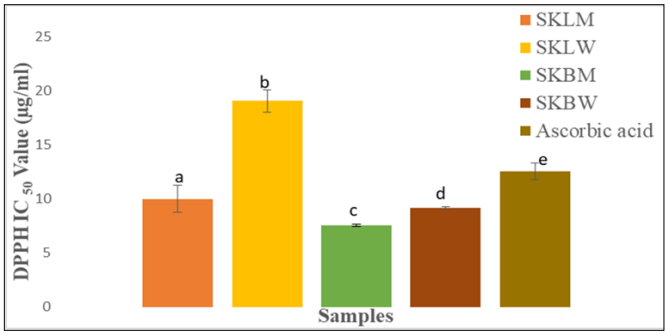

Fig.1. DPPH radical scavenging activity. Values are the mean (n=3) ± Standard deviation, where different alphabets represent significance among samples, p<0.05 in columns. **SKLM**- *Syzygium kanarense* leaf methanol extract; **SKLW**- *Syzygium kanarense* leaf water extract; **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract.

In vitro antidiabetic activity

Both SKBM and SKBW extracts had significant α-amylase- and α-glucosidase-inhibition activity. The IC_{50} for the methanol extract was 51.26 ±0.74 µg/mL and 3.82 ±0.02 µg/mL for α-amylase and α- glucosidase respectively (Fig. 2). The activity was significantly ($p<0.05$) higher than the standard acarbose (IC_{50} of α-glucosidase 13.89±0.62 µg/mL).

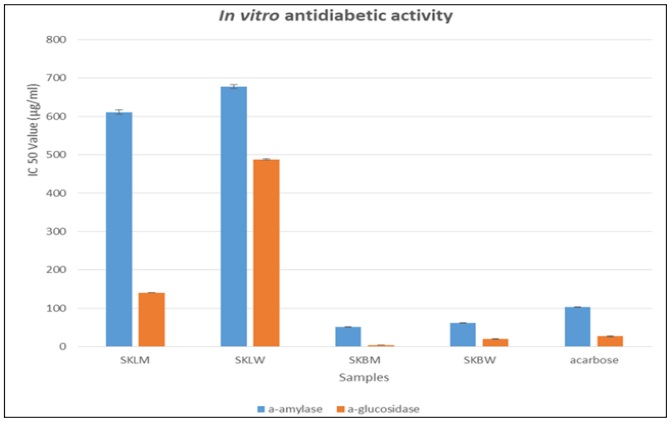

Fig. 2. α-amylase and α-glucosidase inhibition activity. Values are the mean (n=3) ± Standard deviation, where different alphabets represent significance among samples and standard, p<0.05 in columns. **SKLM**- *Syzygium kanarense* leaf methanol extract; **SKLW**- *Syzygium kanarense* leaf water extract; **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract.

Animal studies

Acute oral toxicity study

The treated animals did not show any significant physical or behavioural changes in the initial 4 hrs. Motor activity, food and water intake were unaffected. Additionally, there were no salivation, aggression, diarrhoea and skin irritations in the 14 consecutive days of observation. There was no mortality at the fixed dosage of 2000 mg/kg body weight.

Oral glucose tolerance test (OGTT)

The oral administration of SKBM and SKBW extracts enhanced glucose tolerance in oral glucose-loaded rats

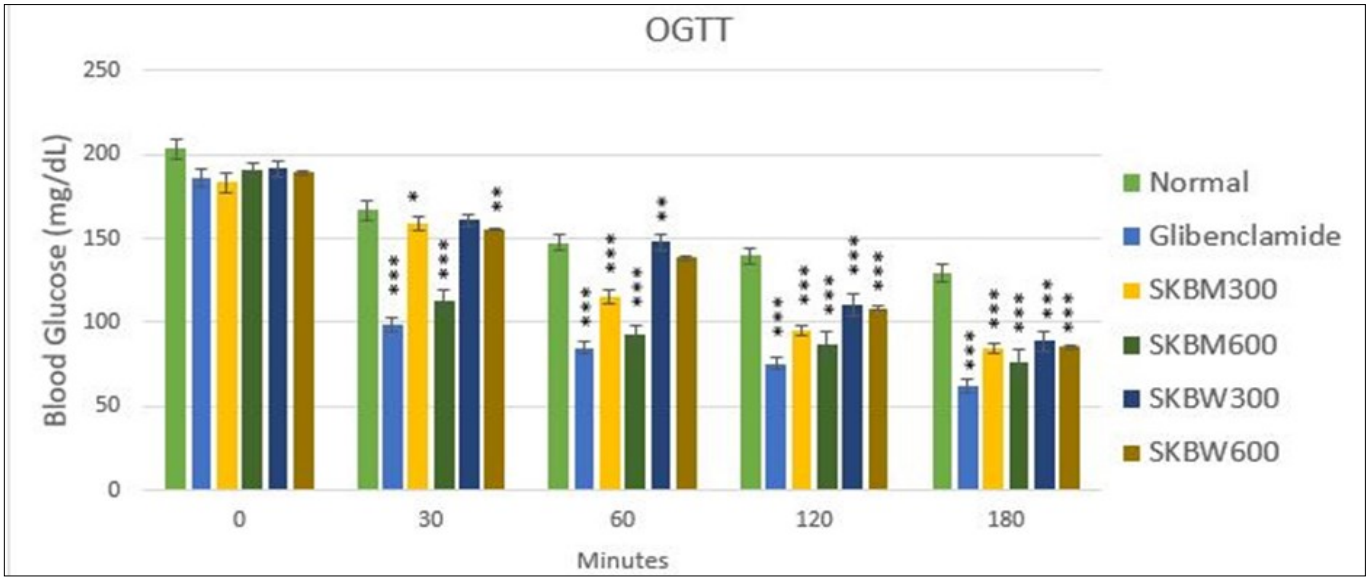

Fig. 3. Oral glucose tolerance in rats treated SKBM and SKBW. Values are the mean (n=4) ± Standard deviation, significantly different at *p<0.05, **p<0.01 and ***p<0.001, when compared to normal. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract.

(Fig. 3). After an initial spike, the glucose level was significantly reduced. Compared to the extract-treated groups, sudden hypoglycaemia was observed in the glibenclamide-treated group at 30 min (98.33±4.22 mg/dL). 600 mg/kg of SKBM showed a considerable decrease in the first hr. after treatment (93±5.65 mg/dL). Both the lower (300 mg/kg) and higher (600 mg/kg) doses of both extracts brought blood glucose levels to normal after 180 min, indicating the extracts' potential to provide glucose tolerance. The extracts show a steady and gradual decrease in the glucose level in comparison to the glibenclamide-treated group.

Assessment of antihyperglycemic activity

Both extracts showed significant antihyperglycemic activity throughout the experimental period. In comparison to the other groups, SKBM and SKBW (600 mg/kg) were found to be more effective in lowering blood glucose levels ($p < 0.001$). The SKBM and SKBW extracts induced weight gain during the study period (Table 3 & Table 4).

Effect on biochemical parameters

The effect of SKBM and SKBW on biochemical parameters is given in Table 5. The level of oxidative stress marker enzymes AST and ALT were significantly ($p<0.05$) lesser compared to the diabetic control group. Also, a significant ($p<0.001$) enhancement in catalase activity was observed in extract-treated groups. The albumin (Alb) and total protein (TP) levels in the serum were comparable to the normal rats. There was a significant ($p<0.001$) increase in creatinine and urea levels in the diabetic control group compared to the normal group, whereas these were markedly reduced in Glibenclamide, SKBM (600 mg/kg), SKBW (600 mg/kg) with $p<0.001$ and SKBM (300 mg/kg), SKBW (300 mg/kg) with $p<0.05$ treated groups.

Effect on lipid profile

Diabetic rats treated with higher concentrations (600 mg/

Table 3. Effect of SKBM and SKBW on blood glucose

Groups	Blood glucose concentration (mg/dL)					
	Initial	On 0th day	On 5th day	On 10th day	On 15th day	On 21st day
Normal	92.33±4.18	93.17±2.79 a	91.33±3.61 a	92.33±3.27 a	90.17±1.72 a	90.67±3.78 a
Diabetic	87.67±3.83	401.33±6.15 b	469.17±15.43 b	506.5±18.66 b	537.16±25.83 b	546.33±22.60 b
Glibenclamide	79.50±4.77	425.67±16.81 c	329.83±13.71 c	222.33±10.98 c	145.67±22.17	94.67±2.80 a
SKBM(300)	83.66±5.58	398.17±10.74 b	364.16±11.51 cd	320.33±14.52 d	288.69±14.46 d	249.00±23.50 c***
SKBM(600)	92.50±3.28	401.17±13.76 b	303.17±6.52 ce	266.33±5.99 e	192.50±8.26 e	125.83±4.45 d***
SKBW(300)	91.00±5.76	406.00±8.67 b	334.00±11.37 c	294.80±5.59 f	253.67±26.01 f	200.17±7.22 e***
SKBW(600)	96.17±7.99	409.00±24.81 b	331.00±28.56 c	283.67±15.04 f	238.17±15.04 f	132.50±7.31 d***

Values are the mean (n=6) ± Standard deviation, significantly different at *p<0.05, **p<0.01 and ***p<0.001, when compared to diabetic control. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract

Table 4. Effect of SKBM and SKBW on body weight

Groups	Body weight (g)					
	Initial	On 0th day	On 5th day	On 10th day	On 15th day	On 21st day
Normal	193.33±8.78	194.83±8.11 a	196.00±6.69 a	197.00±7.38 a	199.67±6.35 a	205.00±5.55 a
Diabetic	190.17±8.09	173.67±4.03 b	160.17±7.96 b	153.00±7.16 b	152.50±7.89 b	156.83±8.01 b
Glibenclamide	186.67±2.94	167.17±4.31 b	185.83±5.64 a	205.00±5.59 a	216.17±5.71 ac	222.67±5.92 ac
SKBM(300)	207.50±4.23	202.67±18.84 a	190.00±18.43 a	187.5±16.97 a	192.00±14.90 a	198.10±12.15 a***
SKBM(600)	198.00±9.27	190.50±6.35 a	178.17±7.60 ac	183.00±8.44 a	186.50±6.09 ad	196.33±7.39 a***
SKBW(300)	204.17±5.46	196.33±6.44 a	199.17±6.31 a	202.33±5.99 a	208.50±6.38 a	209.33±4.13 a***
SKBW(600)	212.17±5.49	204.67±4.59 a	210.00±3.85 ad	212.33±2.16 a	213.67±2.66 ac	218.33±2.65 ad***

Values are the mean (n=6) ± Standard deviation, significantly different at *p<0.05, **p<0.01 and ***p<0.001, when compared to diabetic control. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract

Table 5. Effect of SKBM and SKBW on biochemical parameters

Groups and Dose (mg/kg)	AST(U/L)	ALT (U/L)	Catalase (µmole H_2O_2 Consumed/mg of protein)	TP (mg/dL)	Alb (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Normal	74.00 ± 3.69	39.83 ± 6.08	31.47±1.46	5.87 ± 0.52	3.73 ±0.27	33.83 ± 2.99	0.32 ± 0.008
Diabetic	137.20 ± 8.12	103.00 ± 4.23	8.27±1.02	7.82 ± 0.85	2.26 ± 0.31	70.40 ± 12.37	0.66 ± 0.08
Glibenclamide(5)	87.80 ± 11.92	44.40 ± 3.01	23.46±0.37	5.84 ± 0.12	2.80 ± 0.18	31.00 ± 7.40	0.54 ± 0.08
SKBM(300)	107.60 ± 3.97*	66.60 ± 3.36***	22.56±1.28***	6.78 ±0.34**	2.65 ± 0.22**	48.83 ± 5.23***	0.53 ± 0.05*
SKBM(600)	103. 20 ± 3.90***	46.40 ± 6.66***	26.80±1.68***	4.92 ± 0.63***	3.18 ± 0.23***	44.80 ± 6.46***	0.42 ±0.11***
SKBW(300)	121.80 ± 6.38*	86.60 ± 16.10*	15.88±1.02***	5.80 ± 0.97***	2.94 ± 0.11***	84.20 ±17.63*	0.54 ±0.05*
SKBW(600)	101.40 ± 8.88***	53.00 ± 5.15***	33.26±0.18***	5.80 ± 0.30***	3.02 ± 0.13***	39.60 ±5.59***	0.38 ±0.08***

Values are the mean (n=6) ± Standard deviation, significantly different at *p<0.05, **p<0.01 and ***p<0.001, when compared to diabetic control. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract

kg) of extracts showed similar effects as Glibenclamide-treated rats. Similarly, when compared to the diabetic rats, a significant ($p<0.001$) decrease in TC, TG, LDL and VLDL levels was noticeable in the extract (600 mg/kg) treated rats. Comparing diabetic rats to normal control rats, there was no difference in HDL levels (Fig. 4).

Histopathological studies

The photomicrograph of the histopathology of the liver is illustrated in Fig. 5. The STZ+ NA-induced diabetic rats showed necrotic and distorted hepatic cells with dilated

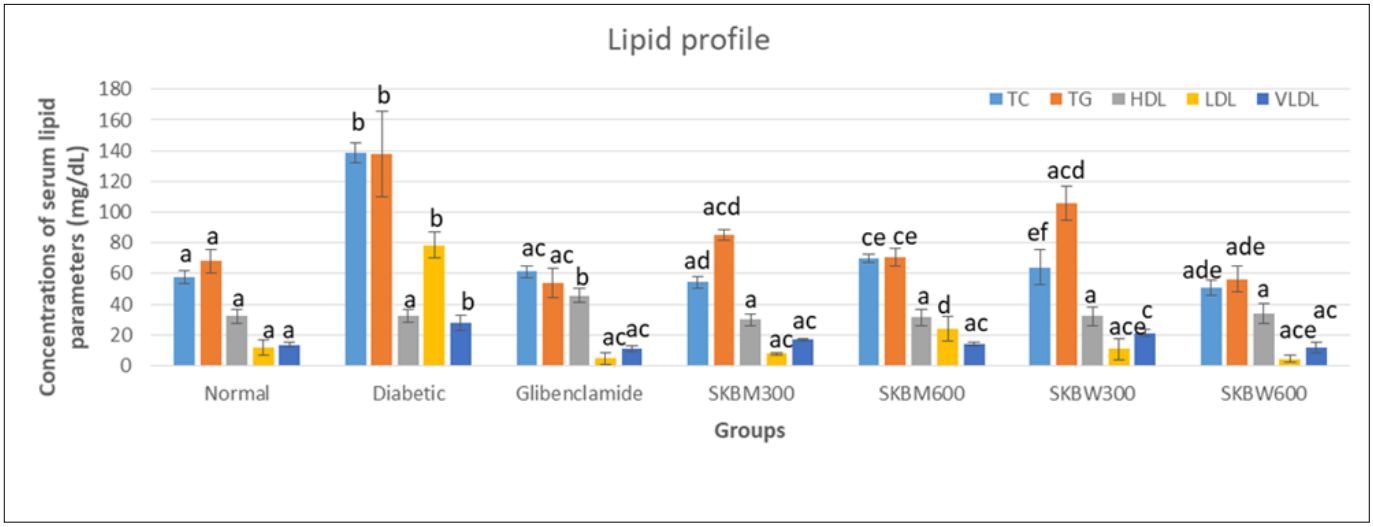

Fig. 4. Effect of SKBM and SKBW on lipid profile. Values are the mean (n=6) ± Standard deviation, significantly different at $p<0.001$, when compared to diabetic control. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract. **TC**- Total cholesterol, **TG**- Triglycerides, **HDL** – High density lipoprotein, **LDL**- Low density lipoprotein**, VLDL**- Very low-density lipoprotein.

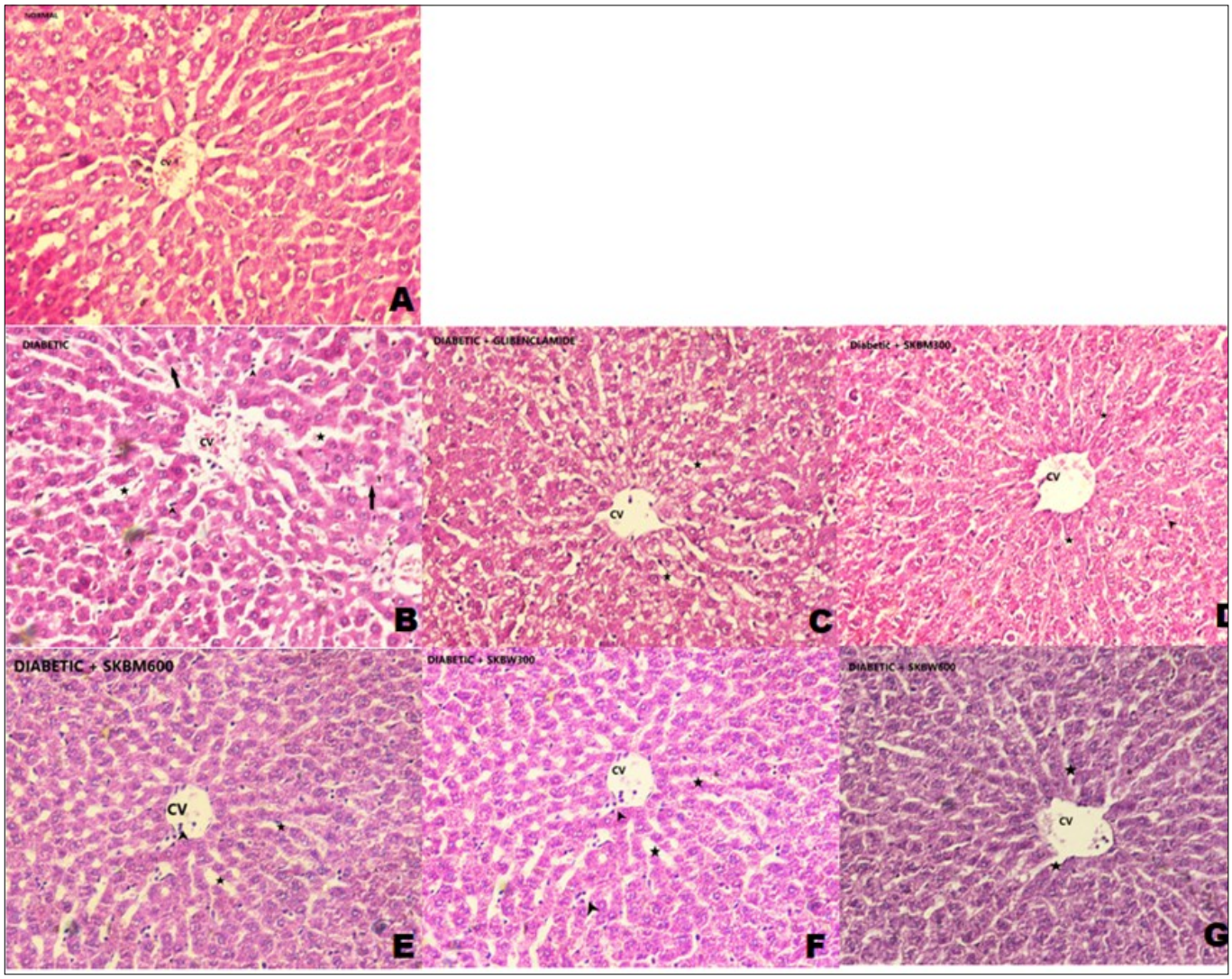

Fig. 5. Effect of SKBM and SKBW on liver histopathology. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract; **CV**- Central vein. Pointed arrow shows necrosis of hepatocytes, arrow heads show Kupffer cells and the star represents sinusoids. Scale - 0.2 mm. (**A**) Normal control, (**B**) Diabetic control, (**C**) Diabetic + Glibenclamide (5 mg/kg), (**D**) Diabetic + SKBM (300 mg/kg), (**E**) SKBM (600 mg/kg), (**F**) SKBW (300 mg/kg), (**G**) Diabetic + SKBW (600 mg/kg).

sinusoids. Whereas the diabetic rats treated with *Syzygium kanarense* extracts showed improved liver architecture with normal hepatocytes arranged in cords around the central vein with mild dilation of sinusoids. A similar result was observed in diabetic rats receiving glibenclamide treatment. The diabetic rats showed deformed glomeruli, degenerated Bowman's capsule and fat deposition in the medullary region (Fig. 6). The normal control rats showed a normal kidney architecture with a narrow Bowman's space and a clear demarcation of proximal and distal tubules without any fat deposition. The diabetic rats that received a high dose (600 mg/kg) of extracts showed glomeruli regeneration and had no fat accumulation. The glomeruli in diabetic rats treated with a lower dose (300 mg/kg) of the extracts were recovered, displaying slightly widened Bowman's space and little fat accumulation. The normal control group also exhibited comparable histopathological conditions. A localized reduction in the size of pancreatic islet size and severe reduction in the beta cells were observed in STZ+ NA administered rats

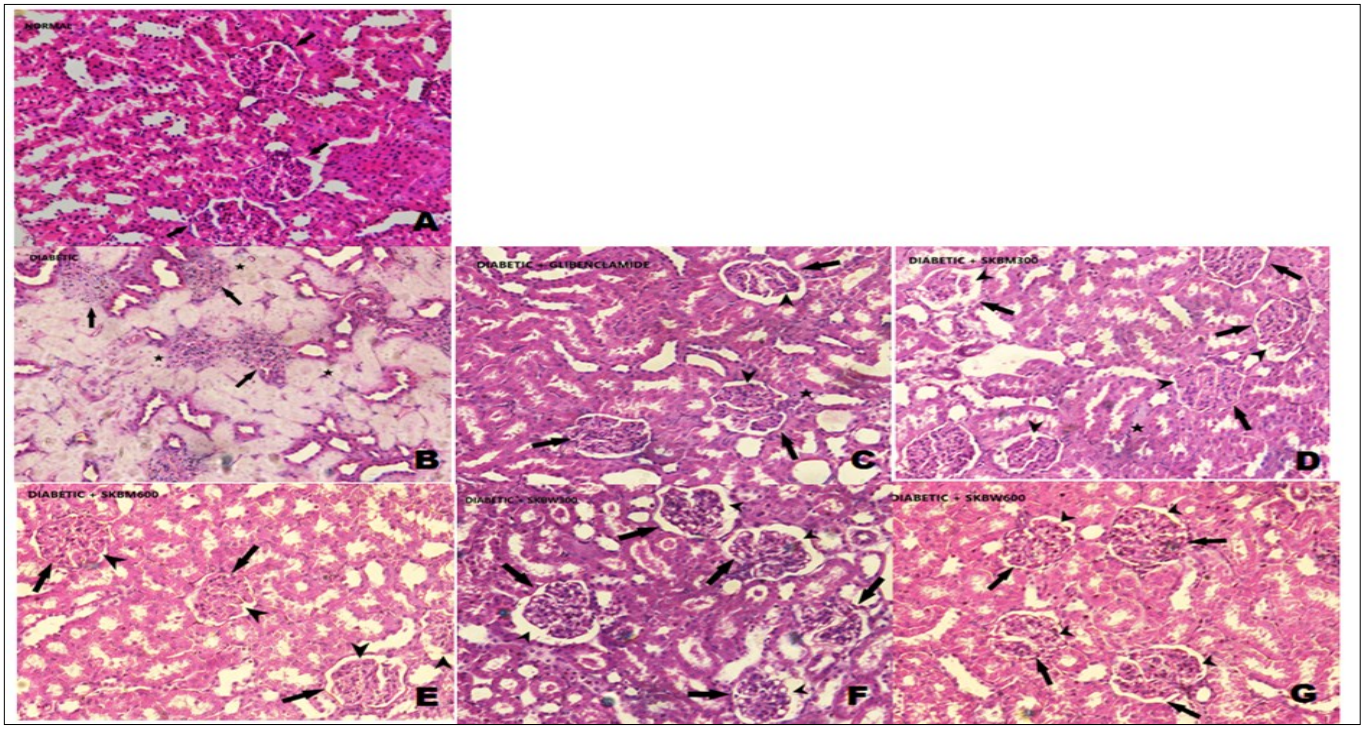

Fig. 6. Effect of SKBM and SKBW on kidney histopathology. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract. Pointed arrows show glomeruli and arrowheads show Bowman's space and the stars show deposition of fat in the medullary region. Scale - 0.2 mm. (**A**) Normal control, (**B**) Diabetic control, (**C**) Diabetic + Glibenclamide (5 mg/kg), (**D**) Diabetic + SKBM (300 mg/kg), (**E**) SKBM (600 mg/kg), (**F**) SKBW (300 mg/kg), (**G**) Diabetic + SKBW (600 mg/kg).

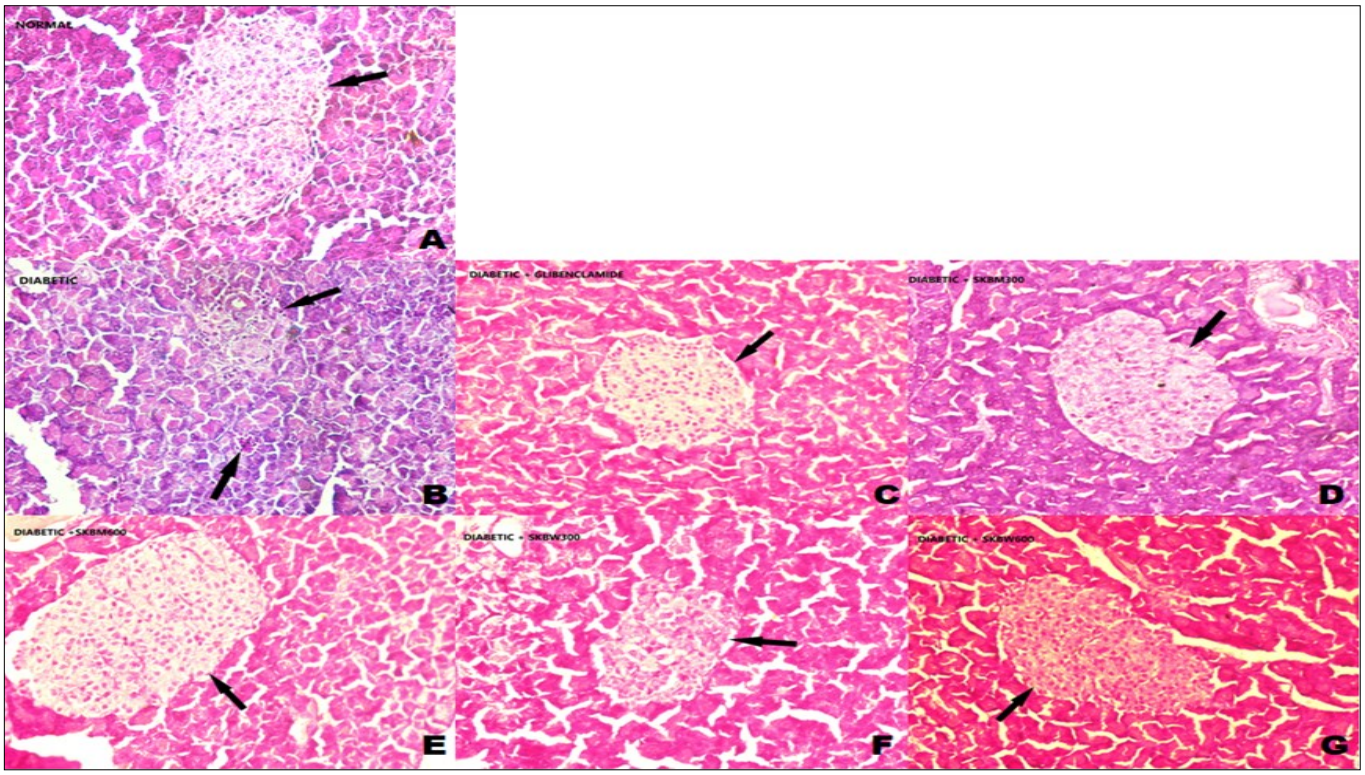

Fig. 7. Effect of SKBM and SKBW on pancreas histopathology. **SKBM**- *Syzygium kanarense* bark methanol extract; **SKBW**- *Syzygium kanarense* bark water extract. The pointed arrow shows Islets of Langerhans. Scale - 0.2 mm. (**A**) Normal control, (**B**) Diabetic control, (**C**) Diabetic + Glibenclamide (5 mg/kg), (**D**) Diabetic + SKBM (300 mg/kg), (**E**) SKBM (600 mg/kg), (**F**) SKBW (300 mg/kg), (**G**) Diabetic + SKBW (600 mg/kg).

(Fig. 7). Glibenclamide (5 mg/kg) treatment in diabetic rats led to the potential regeneration of islets and an increase in the number of beta cells. Similar results were observed in diabetic rats treated with a lower dose (300 mg/kg) with restored acinar cells. The islets of Langerhans regenerated remarkably in diabetic rats receiving 600 mg/kg of the extract with numerous evenly distributed beta cells.

Discussion

Secondary metabolites in plants, such as phenolic substances, protect cells from oxidative stress and have a substantial impact on antihyperglycemic activity (24). Flavonoids like quercetin and myricetin extracted from various species of *Syzygium* have been shown to rejuvenate pancreatic cells in diabetic rats (25) and improve insulin signaling, particularly in type 2 diabetes (26). The presence of different phytochemicals was confirmed, with phenols and flavonoids being the most abundant in *Syzygium kanarense* bark extracts compared to leaf extracts. The findings are consistent with other species of *Syzygium* (27).

A shift in the balance between oxidants and antioxidants in cells and tissues causes oxidative stress resulting in biological damage which is one of the primary causes of diabetes (28). *Syzygium* spp. is well-known for its antioxidant activity. Well-studied antioxidant polyphenolic compounds like catechin, gallic acid, myricitrin and quercitrin have been isolated from various species of *Syzygium* (29, 30) In this study all the extracts tested had exceptional antioxidant activity.

One of the approaches for high blood sugar treatment is to block carbohydrate breakdown in the digestive tract. As a result, in diabetes, controlling postprandial blood glucose level is critical. Herbal materials have been tested for their ability to inhibit the polysaccharide breakdown enzymes like α -amylase and - α –glucosidase (31). This, in turn, is a measure of their antidiabetic activity. Compared to the usual medication acarbose, SKBM and SKBW extracts exhibited significant inhibition of these enzymes. This shows the efficacy of extracts in reducing carbohydrate digestion (32).

An acute toxicity test with 2000 mg/kg of the extract did not show any metabolic anomalies or behavioral changes. Many of the *Syzygium* spp. studied did not show any toxic response (33).

Streptozotocin- nicotinamide (STZ- NA) administered diabetic rat model is the preferred model to examine the antihyperglycemic action of various drugs in type 2 diabetes. This model is experimentally amenable since it maintains mild hyperglycemia and moderately decreased β-cell mass with medium insulin secretion (34). After 21 days of treatment, the extracts were found to lower the blood glucose levels in STZ- NA-induced diabetic rats and their efficiency was proportional to their concentration. The polyphenols present in the bark extracts may be responsible for this (35). The extract-treated rats regained their body weight from an initial loss. This might be because the animals were capable of utilizing glucose instead of proteins as the energy source (36).

The enzymes AST and ALT serve as markers for oxidative stress-linked hepatocellular injury (37). Patients with Type- 2 diabetes have elevated levels of transaminases. ALT level inversely correlates with suppressed insulin production (38). In our investigation, diabetic rats treated with the extracts showed decreased AST and ALT levels, comparable to the glibenclamide-treated rats. A heme protein catalase plays an integral role in eliminating oxidative stress. The production of reactive oxygen species (ROS) in STZ-NA-induced rat models is accompanied by increased blood sugar. The increased glucose glycates the catalase rendering it non- functional (39). An increased catalase activity is seen in extract- treated groups.

Total protein, albumin and creatinine levels are employed as markers for kidney health (40). In contrast to normal control rats, diabetic rats had lower concentrations of albumin and higher concentrations of urea and creatinine in their serum. This skewed profile in the diabetic population illustrates how diabetes accelerates renal dysfunction brought about by cellular damage (41). The groups treated with SKBM (300 mg/kg, 600 mg/kg) and SKBW (600 mg/kg) had decreased levels of urea and creatinine. This suggests that *Syzygium kanarense* had a beneficial impact in restoring renal impairment.

In both clinical and experimental diabetes, hyperlipidemia is the most common metabolic consequence (42). An increase in HDL-C is a very desirable biochemical condition that reduces the risk of heart disease (43). Reduced lipoprotein lipase activity, whose production is stimulated by insulin, may be the cause of elevated TG in diabetic rats (44) and TC elevation may be caused by reduced hepatic cholesterologenesis and enhanced intestinal cholesterologenesis (45). In our study, the STZ+NA treated rats showed a significant rise in TC, TG and LDL- C levels. These were significantly lowered after the extract treatment which was near normal. Polyphenols like flavonoids, glycosides and tannins may contribute to the reduced activity of 3-hydroxy-3-methylglutaryl-coenzyme, which controls the serum lipid profile (46). The level of HDL-C was raised in the extract-treated groups. It can, therefore, be inferred that the bark extract has the ability to lessen the long-term consequences of diabetes.

Diabetes promotes the glycosylation of proteins causing anomalies in hepatic histoarchitecture (47). An increased presence of Kupffer cells (Fig. 5) in the liver indicates the structural damage brought about by diabetes (48). A correlation between the morphological abnormalities in the kidney and raised serum creatinine and urea level was observed in diabetic rats, which indicates decreased renal function. Treatment with the extracts significantly improved the pathological changes in the liver and kidney. The number of ß-cells and size of the islets are crucial factors in determining the severity of pancreatic injury (49). In diabetic rats, islets were small and withered, and the beta cells were destroyed. After SKB therapy, well-formed islets and an increase in ß-cells were seen which

could lead to increased insulin activity. In addition to its ability to lower blood sugar levels, histological findings support the idea that *Syzygium kanarense* shields the liver, kidneys and pancreas from STZ+NA-induced damage in diabetic rats. These results are in good agreement with earlier investigations, which described comparable histological alterations after STZ + NA-induced diabetes (26, 50).

From the results of *in vitro* antioxidant and enzyme inhibitory studies, it was evident that the bark extracts were more potent than the leaf extracts and were further evaluated for *in vivo* antidiabetic activity. Both the extracts showed comparable antidiabetic activity in a dose-depen dent manner. Major components in both the extracts are polyphenols which are well- known antioxidants and possibly this could be responsible for the antidiabetic activity.

Conclusion

The bark extracts of *Syzygium kanarense* were evaluated using STZ- NA-induced diabetic rat model. Oral administration of the extract (2000 mg/kg) did not result in any observable behavioral changes. The study suggests that both methanol and aqueous extracts of bark exhibit dose-dependent antihyperglycemic activity. In histology, the extracts showed a protective effect on the pancreas, kidney and liver. The extracts exhibited reduced activity of α -amylase and α –glucosidase. Further studies are needed to identify the antidiabetic and antioxidant compounds in the bark extracts.

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Authors contributions

SCH carried out the experiments and drafted the manuscript and KKG designed the experiments and investigate the experiments. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: As per the direction of the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), Government of India, SDM Centre for Research in Ayurveda & Allied Sciences, SDM College of Ayurveda Campus, Udupi, Karnataka, India has approved the protocol (Ref.No: SDMCRA/IAEC/MU-09 dated 07/01/2019) to carry out the experiments on rats.

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