

Biochemical Study on the Anti-Hyperglycemic Effects of Coconut Testa (*Cocos nucifera* L.) and Red Kidney Bean (*Phaseolus vulgaris*) Seed Coat in Streptozotocin-Induced Diabetic Rats

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Abstract

Proper management of hyperglycemic condition is the hallmark in the treatment of diabetes mellitus. As plants with good antioxidant potentials are known to have beneficial effect on diabetes and related complications, we aimed at comparing the anti-hyperglycemic activities of the extracts of coconut testa (TOC) and red kidney bean (RKB) seed coat. In this study, the effect of RKB and TOC extracts on Streptozotocin-induced diabetic Sprague-Dawley rats was investigated. Forty-nine male rats were randomly divided into seven groups having seven rats each for normal control, diabetic, and diabetic-treated groups. The oral administration of the two extracts was carried out daily for 14 days at dose levels of 200 and 400 mg/kg b.wt. The anti-hyperglycemic effect of the two extracts were determined using blood glucose levels withdrawn from the animal's tails after administering glucose at 0, 30, 60 and 120 min. The anti-diabetic effects of the extracts were determined using fasting blood glucose levels after 72 h of injection of extracts. The blood parameters such as cholesterol, urea, bilirubin, creatinine, total protein, Alanine transaminase (ALT), and Aspartate transaminase (AST) were determined using serum isolated from overnight-fasted animals after 14 days of treatment. Results indicated that both TOC and RKB extracts at different doses were capable to reduce hyperglycemia condition significantly ($p < 0.05$). TOC demonstrated the most remarkable reduction at the dose of 200 mg/kg b.wt. Treatment of these two extracts brought significant improvements in some of the blood parameters tested. This study concludes that RKB seed coat and TOC would have high potential to be used as natural sources to prepare food products for diabetic management.

Keywords

Antihyperglycemia, Red kidney bean, Coconut testa, Biochemical study, Streptozotocin

Abbreviations

DOA: Department of Agriculture; RKB: Red kidney beans; STZ: Streptozotocin; TOC: Testa of coconut; ALT: Alanine transaminase; AST: Aspartate transaminase; SD: Standard deviation; ANOVA: Analysis of Variance

Introduction

Diabetes mellitus (DM) is an endocrine disorder of carbohydrate, protein, and lipid metabolism characterized by hyperglycemia (increasing of circulating blood glucose), which occurs as a result of defect in insulin secretion, insulin action or both [1, 2]. During the latter part of 2020, an estimated 537 million adults aged

between 20–79 years worldwide (10.5% of all adults in this age group) are living with diabetes. By 2030, 643 million, and by 2045, 783 million adults aged 20–79 years are projected to be living with diabetes [3]. Diabetes mellitus is commonly associated with several complications which can be divided into microvascular (retinopathy, nephropathy, neuropathy) and macro vascular (heart diseases, stroke, peripheral vascular disease) [3]. Dysfunction and failure of different organs such as eyes, kidneys, heart, nerve, and blood vessels are the result of chronic hyperglycemia of diabetes [3]. Failure to arrest the rising trend of diabetes in the country would negatively impact the health of the nation, social welfare of the people, and economic status due to diabetic complications that might ensue.

Obesity, inactive lifestyles, poor eating habits, aging, and genetic predisposition are generally considered as the risk factors of diabetes [3]. Nutritional and dietary interventions can play a greater role in managing hyperglycemia condition among diabetes. Reduction of the intake of carbohydrate foods made with refined wheat flour has been found to be an effective approach in controlling post-prandial hyperglycemia [4]. For this, increase in the consumption of beans of grain legumes is said to be remarkably helpful as they are excellent sources of protein and dietary fibre and provide many essential vitamins and minerals [5]. According to Romero-Arenas *et al.* [6], common beans are cultivated and consumed throughout the world as they are excellent source of protein, carbohydrate, soluble fibers, vitamins (thiamine, niacin, riboflavin, etc.), and minerals (calcium, iron, etc.). These nutritional attributes have been confirmed by the Agriculture Department of Sri Lanka who compiled nutritional information of various varieties of beans grown in the country [7]. Apart from general nutrition, beans are also known to possess high amount of bioactive compounds such as anthocyanins, tocopherols, carotenoids, etc [8]. As such, consumption of whole grains and cereals is recommended as a diet to prevent chronic diseases such as diabetes and cancer [4-5].

Various research groups have investigated the use of alternative flour types made out of millet, green beans, tiger-nuts, soy, and coconut for the above said purpose. In a recent study, Marasinghe *et al.* [9] reported that the flour made out of TOC is a nutrient-rich by-product composed of proteins, carbohydrates, lipids, fibers, minerals, and trace elements such as phosphorus, potassium, magnesium, calcium, and manganese. Despite these merits, utilization of TOC in food formulation is still not popular among the main stream public possibly due to lack of promotional campaign and scarcity of information related their therapeutic values. According to a preliminary screening of TOC and selected beans such as red kidney bean, black-eyed pea, red bean, and black beans of their antioxidative and antihyperglycemic potentials, both TOC and RKB were found to have high antioxidative properties and anti-hyperglycemic activity [5]. Nevertheless, further exploration of these findings using an animal-based *in vivo* study was not previously undertaken to confirm the above mentioned claims. Hence, the objective of this study was to test the anti-hyperglycemic potential of the extracts of TOC and RKB on normo-glycemic and streptozotocin-induced diabetic rats.

Materials and Methods

Materials

Source of raw materials

Samples of RKB (*Phaseolus vulgaris*) were collected in triplicate from a farm in Sitiawan, Malaysia while samples of TOC were collected in triplicate from flesh of coconuts (*Cocos nucifera* L) plucked from a farm located in Seri Kembangan, Malaysia. Plants (RKB: 11200 & TOC: 22300) were cross-checked by a botanist and the voucher specimen were deposited in the Herbarium of University Agricultural Park, University Putra Malaysia.

Pretreatment

The bean samples were soaked for 24 hours and dehulled manually while samples of TOC collected from the flesh of coconut kernel were dried and powdered into fine particles. Prior to the experiment, samples were kept in air tight Ziploc plastic bags and stored at 4 °C.

Preparation of cold ethanol plant extracts

Powdered samples of TOC and RKB seed coats 100 g each were weighed separately and subjected to extraction with a solvent mixture containing 700 mL ethanol and 300 mL water (ethanol: water = 70:30) for 48 hr at room temperature. The extracts were centrifuged with eppendorf 8510R centrifuge at 8300 rpm for 10 min and filtered with Whatman No.1 filter paper. The solvent was evaporated under reduced pressure using a Büchi Model R-205 rotary evaporator (Büchi, Switzerland) and the semi-solid extracts were freeze-dried using Virtis bench top profreeze dryer SJIA-10N (FD-1B-50) (Virtis, New York). The extracts were then re-dissolved in 10 mmol citrate buffer (vehicle) for pharmacological experiments.

Experimental animals

For this study, male Sprague-Dawley rats (150–180 g) with no prior drug treatment were supplied by A-Sapphire Enterprise, Selangor D.E., Malaysia. Prior to the commencement of the experiment, all animals were allowed to acclimatize to their new environmental conditions for a week. The animals were fed with standard rat chow and water *ad libitum*. Room temperature of 26 ± 2 °C with 12-h day/night cycle and 45–55% of relative humidity were maintained.

Ethical consideration

Experiments of this study were performed in accordance with Malaysian Guidelines for Good Clinical Practice. The Institutional Animal Care and Use Committee (IACUC) of Universiti Putra Malaysia (UPM) approved the experimental protocol, which was given the reference number; UPM/IACUC/AUP-R041/2016.

Study of anti-hyperglycemic activity

The method described by Sangeetha *et al.* [10] was used with some minor modifications to study the anti-hyperglycemic activity in glucose-loaded hyperglycemic animals. The animals were randomly divided into six treatment groups (each group containing seven animals; n=7)

and administered the extracts of TOC and RKB at different doses. They were orally treated in the following manner: Group I: Normal control rats received distilled water only (10 mL/kg b.w); Group II: Animals treated with RKB200 mg/kg b.wt; Group III: Animals treated with RKB400 mg/kg b.wt; Group IV: Animals treated with TOC200 mg/kg b.wt; Group V: Animals treated with TOC400 mg/kg b.wt.; Group VI: Animals treated with Glibenclamide (10 mg/kg b.wt.). After 30 min of oral dosing, the animals were fed with glucose (2 g/kg b.w.) orally using gastric intubation. Blood samples were withdrawn from the animal's tail at 0, 30, 60 and 120 min for glucose administration. In all these experiments, approximately 0.5 ml blood was withdrawn each time from the tail using aseptic precautions and the serum was separated immediately by centrifuging at 3000 rpm for 15 min. The glucose concentration in the serum samples was analyzed immediately by the glucose oxidase method using Randox assay kit (Randox Laboratories Ltd., Co. Antrim, UK) and a spectrophotometer (V500 Jasco Cooperation, Tokyo, Japan). Water was used as a vehicle since all samples were soluble in water.

Study of anti-diabetic activity

Type 2 diabetes was induced in overnight fasted Sprague-Dawley rats by a single intraperitoneal injection of freshly prepared streptozotocin at a dose of 45 mg/kg b.wt. in 10 mmol citrate buffer (pH 4.5). The method described by Sangeetha et al. [10] with some modification was used to study the anti-diabetic activity of the two extracts. The diabetic animals were divided into seven groups of seven animals each and administered with TOC and RKB extracts at different concentrations as shown here. They were orally treated in the following manner: Group I: Normal control rats received vehicle (10 mL/kg b.w); Group II: Diabetic control rats received vehicle (10 mL/kg b.w); Group III: Diabetic treated rats received RKB 200 mg/kg b.wt; Group IV: Diabetic treated rats received RKB 400 mg/kg b.wt; Group V: Diabetic treated rats received TOC200 mg/kg b.wt; Group VI: Diabetic treated rats received TOC400 mg/kg b.wt; Group VII: Diabetic treated rats received Glibenclamide (10 mg/kg b.wt). The animals in the normal and control groups received only citrate buffer as vehicle. Glucose solution (10% w/v) bottles were kept in their cages for the next 24 h to prevent hypoglycaemia. Fasting blood glucose levels was determined after 72 h of injection. The animals showing fasting blood sugar of 300 mg/dL or more were considered diabetic and included in the study. The animals were fed daily with TCO and RKB using an intragastric tube for 14 days. The fasting blood glucose measurement was determined on 0th, 3rd, 7th and 14th day by glucose oxidase method with commercially available test kit. The body weight of animals was determined on day 0th and the last day of the study. After the experimental period, the animals were fasted overnight and sacrificed under anaesthesia.

Body weight measurement

Body weights of the animals were monitored on day 0th and the last day of the study. For all experimental days, the weighing time was fixed (8:00 am to 9:00 am).

Determination of blood biochemical parameters

After 14 days of treatment, serum was isolated from the blood collected from overnight fasted animals to determine the biochemical parameters. Blood was withdrawn through cardiac puncture with the aid of sterile syringes and needles. They were then dispensed into sterile tubes. The blood was allowed to clot for few hours after which it was subjected to centrifuging at 3500 rpm for 30 min in order to separate the serum from the whole blood. Serum was removed with sterile needles fixed into syringes and stored in the freezer until further analysis. Serum total protein, total cholesterol, blood urea, bilirubin and creatinine were estimated by standard enzymatic methods using Hitachi 902 automated biochem analyzer. Serum levels of aspartate aminotransferase and alanine aminotransferase were determined by using diagnostic colorimetric kits and measured spectro-photometrically.

Statistical analysis

All measurements were carried out in triplicate (n = 3) and data were presented in the form of mean \pm standard deviation (SD). Minitab software package (version 16.0) was used for all statistical analysis. Two-ways ANOVA followed by Bonferroni posttest was performed for normoglycemic, oral glucose tolerance test and evaluation of blood glucose of STZ-induced diabetic rats. One-way (ANOVA) was applied for the statistical analysis of the other parameters. When *p* values were significant, mean differences were compared using Tukey's multiple range test at 5% level of probability.

Results and Discussion

Anti-hyperglycemic activities of extracts

The blood glucose levels measured at different time intervals (0, 30, 60 and 120 min) after oral administration of glucose (2g/kg body wt.) are as given in table 1. The aim of the glucose-tolerance test was to see the efficacy of the selected plant extracts to counter the increasing blood glucose after a glucose load without tempering with the pancreatic islets. Before administering the extracts, there was no significant ($p > 0.05$) difference in the blood glucose levels among all groups. However, after 30 min of administration of 2 g/kg glucose, significant ($p < 0.05$) rise in the blood glucose levels were noticed among all groups. The spike in postprandial hyperglycemia is a normal physiological phenomenon among people with diabetes, which is an independent risk factor for cardiovascular disease [11]. Maintaining the control over the postprandial blood glucose level by inhibiting the activities of the carbohydrate-hydrolyzing enzymes in the small intestine through drugs is one of the most important approaches in the management of diabetes [12]. Several previous reports have already indicated that consumption of several types of legumes are helpful in lowering glycemic responses by reducing the rate at which carbohydrate is absorbed [4]. According to Table 1, among animal groups treated with different extracts, significant ($p < 0.05$) differences were noticed in blood glucose levels after 120 min of administration. The blood glucose levels of the group treated with Glibenclamide, which is a known antidiabetic drug was significantly ($p < 0.05$) lower than that of any other group. Glibenclamide which is

Table 1: Effect of RKB and TOC extracts on BGL (mg/dL) of glucose-loaded hyperglycemic rats.

Groups	FBG (mg/dl)			
	0 min	30 min	60 min	120 min
Normal control	99.00 ± 1.80 ^a	107.10 ± 3.82 ^a	106.20 ± 6.49 ^a	101.70 ± 3.82 ^a
RKB (200mg/kg b.wt.)	105.75 ± 10.64 ^a	160.80 ± 13.98 ^b	144.00 ± 11.24 ^b	98.5 ± 1.27 ^c
RKB (400mg/kg b.wt.)	98.40 ± 1.04 ^a	161.10 ± 6.36 ^b	134.40 ± 12.72 ^b	94.1 ± 1.27 ^b
TOC (200mg/kg b.wt.)	98.40 ± 1.04 ^a	122.40 ± 4.76 ^c	108.90 ± 6.36 ^a	100.80 ± 5.09 ^c
TOC (400mg/kg b.wt.)	102.60 ± 6.49 ^a	157.60 ± 12.35 ^b	115.20 ± 10.18 ^a	100.80 ± 2.55 ^c
Glibenclamide (5mg/kg b.wt.)	104.40 ± 7.85 ^a	142.90 ± 10.89 ^d	110.60 ± 1.41	79.2 ± 7.64 ^a

Abbreviations: BGL, blood glucose level; RKB, red kidney bean; TOC, testa of coconut. Values were expressed as mean ± SD. n=7.

Different superscripts (a, b, c, d, etc.) on the same column were significantly (p<0.05) different.

^{a,b} P <0.05, compared with normal control values.

also known as glyburide is a sulphonylurea compound that stimulates the release of insulin by blocking ATP-dependent potassium channels K (ATP) in the β -cells of the pancreas [13]. Interestingly, blood glucose level of the group treated with RKB 400 mg/kg b.wt was found to be significantly (p<0.05) lower than that of the negative control group. This study suggested that the improvements in glucose tolerance would be the result of increased secretion of insulin due to the presence of hypoglycemic components in the extracts. In the preliminary *in vitro* screening, Adekola *et al.* [5] showed that the extracts of TOC and RKB had high potencies to partially inhibit the digestion of carbohydrates due to the presence of bioactivity phytochemicals such gallic acid, catechin, epigallocatechin, gallate, chlorogenic acid, and caffeic acid. This finding was further confirmed through a separate study conducted on TOC of different coconut cultivars of Sri Lanka [14]. The bioactive substances occurring in fruits, vegetables, and cereals are generally known to play an important role as antioxidant, anti-inflammation and antihypertension agents. In addition, they have the potential to reduce the organ damages and entail hepato-protective effects in animals [15].

Anti-diabetic activities of extracts

Animal models are frequently employed as tools for assessment of the therapeutic potentials of novel anti-diabetic agents. Although several synthetic drugs are used to induce diabetes in animals, Streptozotocin (STZ) and Alloxan monohydrate have become more prominent due to their efficacy and cost effectiveness. STZ is widely used to induce diabetes in experimental animals since it can act by damaging the insulin secreting β -cells of pancreas thereby leading to an increase in blood glucose levels [16]. Data presented in table 2 shows the effect of a single injection of STZ on changes in fasting blood glucose levels among different diabetic groups. All animal groups displayed the fasting blood glucose within the normal physiological range prior to injecting STZ. Rats in all STZ-induced groups experienced hyperglycemia after three days of the injection; their fasting blood glucose levels were significantly (p<0.05) higher than that of non STZ-induced group. Damages in islets of Langerhans in the pancreas could be a probable reason for the spiking fasting blood glucose

levels observed among diabetic rats [12]. After confirmation of the diabetic status of the animals, the treatment of the animal groups continued until the 14th day of the study (last day). Throughout the course of the treatments, all diabetic groups treated with different doses of the extracts (RKB400, TOC400, RKB200 and TOC200) responded differently. According to the data of the blood glucose levels on the last day of the study, none of the diabetic treated groups had their fasting blood glucose normalized to those of the non STZ-induced group. However, administration of different doses of extracts of RKB and TOC (200 and 400 mg/kg body weight) to the STZ-induced diabetic rats significantly reduced blood glucose level. When compared to the diabetic control group, Glibenclamide treatment showed a strong decrease in fasting blood glucose level as shown in table 2. Among the treatments, TOC of 200mg/kg body weight ameliorated the fasting blood glucose level to the best when compared to normal control as well as the other doses, though it is comparable to fasting blood glucose level of RKB at dose of 400 mg/kg b.wt. This could be probably because the extracts of TOC and RKB would have helped to reduce blood glucose level in the diabetic animals since these plant extracts are rich in polyphenols and flavonoids which are already known to reduce risks of diabetes and bring improvements in glycaemic control [5, 14, 17].

Body weight changes

Body weight changes of normal and STZ-induced diabetic rats were compared before and after treatments as shown in table 3. Animals in all groups did not show any significant (p>0.05) difference in their body weights prior to the induction of diabetes. However, remarkable decreases in body weight of STZ-induced diabetic groups (24.89%) were observed with respect to the normal control group, which exhibited a significant (p<0.05) increase in body weight by 22.30%. According to some previous reports, the process of gradual weight loss in diabetic subjects is due to muscle wasting and proteolysis (breakdown of protein in tissues) [10, 12]. Ojo *et al.* [18] further reported that diabetes usually leads to increases in blood glucose levels which ultimately results in lack of glucose intake by the cells. This will cause weight losses by reduction of fats and proteins in the body

as the cells are forced to utilize fatty acids and amino acids as alternate energy sources. According to table 3, there were significant differences in the % change of body weights when compared to the normal control group. Treatments with RKB at dose of 400 mg/kg and 200 mg/kg were generally found to improve the body weights to certain extent when compared to the diabetic control group. When RKB at 400 mg/kg showing the least percentage decrease of -1.9%, RKB extract at a dose of 200 mg/kg exhibited a higher percentage decrease in comparison to the positive control drug treatment (Glibenclamide). Improvements in animal body weights by RKB extracts indicated that the restrain over muscle wasting occurred as result of glycemic control, thereby suggesting its hypoglycemic effect.

Blood biochemical parameters

The effect of RKB and TOC extracts on the kidney functions were assessed by determining the levels of serum creatinine and urea as they are regarded as important renal function markers [18]. Bilirubin and cholesterol are two other blood parameters, which have some influence on kidney function [19, 20]. It has been already recognized that people with cholesterol problems were twice as likely to have chronic kidney disease over time. Particularly, high total cholesterol or reduced HDL (“good”) cholesterol are more likely to cause reduced glomerular filtration rate (GFR) [19]. Previously Kumar *et al.* [16] stated that diabetes mellitus has been

associated with hyperlipidaemia condition, which causes remarkable changes in the concentration and composition of serum lipid parameter such as cholesterol as observed in the present study. As shown in Table 4, significant ($p < 0.05$) differences were observed with regard to cholesterol level between the normal control and diabetic control groups. With respect to diabetic control group, both Diabetic+TOC (200mg/kg b.wt.) and Diabetic+RKB (200mg/kg b.wt.) groups displayed significantly ($p < 0.05$) low cholesterol levels. As diabetes mellitus is a metabolic disorder which usually triggers increase in the level of serum lipid due to inactivation of lipoprotein lipase which hydrolyses triglycerides. Alterations in the concentrations of lipid parameters among the diabetes subjects could lead to development of vascular disease.

Significant ($p < 0.05$) differences were also noticed between the normal control and diabetic control groups with regard to total bilirubin content (Table 4). However, no significant ($p > 0.05$) changes were observed in bilirubin contents between the normal control group and the groups administered with the extracts. A number of studies have been carried out in the past to investigate the effect of bilirubin content on the risk of diabetic complications, but the results were said to be inconsistent. According to a meta-analysis conducted to determine the relationship between bilirubin concentration and the risk of diabetic complications, there was a negative association between bilirubin concentration and the risk of diabetic compli-

Table 2: Anti-Hyperglycemic effect of RKB and TOC extracts on Streptozotocin-induced diabetic rats.

Groups	Blood glucose level (mg/dl)			
	1 st day	3 rd day	7 th day	14 th day
Normal control	112.30 ± 14.89 ^c	122.7 ± 14.70 ^c	121.95 ± 10.33 ^c	120.24 ± 5.0 ^c
Diabetic control	460.50 ± 56.22 ^a	477.0 ± 20.80 ^a	482.00 ± 18.96 ^a	498.00 ± 52.70 ^a
Diabetic+RKB (200mg/kg b.wt.)	457.00 ± 27.97 ^a	451.80 ± 10.20 ^a	450.70 ± 11.74 ^a	375.60 ± 41.45 ^{ab}
Diabetic+RKB (400mg/kg b.wt.)	355.40 ± 26.7 ^{ab}	302.40 ± 18.19 ^{ab}	301.80 ± 32.20 ^{ab}	213.00 ± 45.4 ^{ab}
Diabetic+TOC (200mg/kg b.wt.)	473.67 ± 24.03 ^a	405.60 ± 33.60 ^a	403.80 ± 43.21 ^a	183.60 ± 36.1 ^b
Diabetic+TOC (400mg/kg b.wt.)	455.67 ± 25.77 ^a	437.40 ± 7.85 ^a	413.10 ± 40.0 ^a	357.20 ± 28.4 ^{ab}
Diabetic+Glibenclamide (5mg/kg b.wt.)	432.0 ± 67.10 ^a	428.50 ± 57.28 ^a	407.40 ± 29.98 ^a	339.60 ± 81.80 ^{ab}

Values were expressed as mean ± SD. n=7. Values bearing different superscripts (a, b, c, etc.) on the same column were statistically significant.

Table 3: Changes in body weight of different rat groups treated with RKB and TOC extracts.

Groups	Body weight (g)		% Change in body weight
	0 th day	14 th day	
Normal control	246.67 ± 16.48	301.67 ± 22.84	+22.30*
Diabetic control	225.00 ± 16.55	169.00 ± 21.21	-24.89*
Diabetic+RKB (200mg/kg b.wt.)	188.29 ± 30.12	182.00 ± 5.66	-3.34
Diabetic+RKB (400mg/kg b.wt.)	220.43 ± 21.00	216.25 ± 29.94	-1.90*
Diabetic+TOC (200mg/kg b.wt.)	221.50 ± 22.56	209.80 ± 28.55	-5.28*
Diabetic+TOC (400mg/kg b.wt.)	207.71 ± 27.21	185.75 ± 37.21	-10.57*
Diabetic+Glibenclamide (5mg/kg b.wt.)	222.80 ± 13.74	217.00 ± 32.01	-2.60

Values were expressed as mean ± SD; n=7. Where * indicates the significant difference ($p < 0.05$) in the % change in body weight.

Table 4: Effect of TOC and RKB extracts on serum Urea, Creatinine, Bilirubin, Cholesterol, AST, and ALT.

Groups	Total protein (g/L)	Urea (mmol/L)	Creatinine (μ mol/L)	Bilirubin (μ mol/L)	Cholesterol (mmol/L)	AST (U/L)	ALT (U/L)
Normal control	37.68 \pm 3.31 ^a	3.32 \pm 0.40 ^a	29.40 \pm 2.97 ^a	3.12 \pm 0.49 ^a	0.64 \pm 0.26 ^a	87.80 \pm 30.90 ^a	34.20 \pm 8.26 ^a
Diabetic control	68.93 \pm 5.42 ^b	9.80 \pm 3.39 ^{bc}	51.67 \pm 5.51 ^{bc}	6.00 \pm 1.56 ^b	1.52 \pm 0.07 ^b	279.40 \pm 26.9 ^d	115.20 \pm 85.69 ^d
Diabetic+RKB (200mg/kg b.wt.)	43.98 \pm 3.73 ^a	6.70 \pm 1.11 ^b	43.00 \pm 14.02 ^b	4.03 \pm 0.33 ^a	0.70 \pm 0.26 ^a	143.0 \pm 39.80 ^b	68.25 \pm 15.86 ^b
Diabetic+RKB (400mg/kg b.wt.)	58.75 \pm 0.35 ^{ab}	8.28 \pm 3.95 ^b	40.50 \pm 13.44 ^b	4.86 \pm 1.52 ^a	1.04 \pm 0.16 ^b	201.50 \pm 74.20 ^c	92.50 \pm 12.02 ^c
Diabetic+TOC (200mg/kg b.wt.)	49.98 \pm 10.42 ^a	7.38 \pm 2.35 ^b	45.00 \pm 7.97 ^b	4.36 \pm 0.80 ^a	0.76 \pm 0.22 ^a	214.00 \pm 57.10 ^{cd}	65.80 \pm 30.47 ^b
Diabetic+TOC (400mg/kg b.wt.)	45.10 \pm 18.55 ^a	5.60 \pm 1.49 ^{ab}	34.00 \pm 2.65 ^a	4.17 \pm 0.75 ^a	1.18 \pm 0.44 ^b	238.70 \pm 45.90 ^{cd}	89.67 \pm 40.41 ^c
Diabetic+Glipenclamide (5mg/kg b.wt.)	53.48 \pm 7.63 ^{ab}	7.07 \pm 1.46 ^b	43.40 \pm 6.73 ^b	4.80 \pm 0.36 ^a	1.02 \pm 0.32 ^b	192.00 \pm 54.80 ^{bc}	80.00 \pm 33.15 ^{bc}

Values were expressed as mean \pm SD. n=7. Different superscripts (a, b, c, etc.) on the same column were significantly ($p < 0.05$) different.

cations [21]. However, Shin *et al.* [20] previously demonstrated that there was a direct relationship between serum bilirubin concentration and kidney function. This has been further supported by a subsequent systematic review and meta-analysis [22], which showed that there was a tendency for individuals with reduced bilirubin concentrations to have a higher risk of chronic kidney disease (CKD).

Serum protein levels are generally affected due to diabetes and associated complications. According to Gul and Rahman [23], there was a general tendency for total serum protein to increase in diabetic patients with retinopathy and diabetic patients without any complications. As shown in Table 4, a significant increase ($p < 0.05$) in total protein concentration was noticed in the serum of diabetic control group when compared to normal control group. Previously Nazki *et al.* [24] also observed that the serum total protein concentrations were found to be significantly increased in newly diagnosed patients of Type 2 as compared to controls. Authors suggested that the increase in serum total proteins could be due to the elevation of acute phase proteins, globulins, fibrinogen and compounded by a decrease in the fractional synthetic rate of albumin due to insulin resistance/deficiency. The total serum protein level, however, has reduced substantially after administration of the two extracts (TOC and RKB). This shows the effect of the two plant extract on some blood parameters.

If kidneys are not able to remove urea from the blood normally, the blood urea nitrogen level might rise above normal. Experimental evidence suggests that higher levels of blood urea may tend to increase insulin resistance and suppress insulin secretion. As shown in table 4, the blood urea concentration of the STZ-induced group was increased significantly ($p < 0.05$) with respect to the normal control group. However, no significant ($p > 0.05$) changes were observed in urea concentrations between the diabetic control group and the groups administered with the extracts. Serum creatinine is yet another important parameter used by medical professionals while assessing the kidney function of diabetic subjects. According to

Table 4, the serum creatinine level of the STZ-induced group was increased significantly ($p < 0.05$) with respect to the normal control group. Among the extracts administered groups, Diabetic+TOC (400mg/kg b.wt.) was found to show the lowest value, which was similar to the value recorded for the normal control group. No significant ($p < 0.05$) differences were noticed between the diabetic control group and those administered with other plant extracts with regard to creatinine values.

Liver Enzymes

Alanine transaminase (ALT) and aspartate transaminase (AST) are essential assays for the diagnosis of liver damages occurring as a result of harmful chemicals or drug toxicity [25]. According to the data presented in Table 4, the diabetic agent STZ has caused significant ($p > 0.05$) increases in the levels of plasma ALT and AST with respect to the normal control group. When compared with the diabetic control group, the groups administered with the two plant extracts showed decreases in the levels of plasma AST and ALT. However, this reduction was not enough to reach that of the normal control group as both extracts with two different doses didn't normalize the level of these intracellular enzymes. This study showed increased levels of AST and ALT which was antagonized by the extracts causing a reduction in the level of these enzymes [17]. Increment of the activities of these enzymes has been suggested to be due to leakage of enzymes from the cytosol of the liver into the blood stream [26].

Conclusion

The extracts of both TOC and RKB exhibited anti-hyperglycemic effect with TOC showing the most remarkable decrease in fasting blood glucose level at a dose of 200 mg/kg b.wt. There have been some improvements in selected blood parameters as both extracts were able to normalize the total bilirubin and cholesterol levels. Significant differences were also observed in the groups administered with the extracts and the normal control group with regard to the urea and creatinine

levels. By overall, TOC and RKB could possibly serve as good sources of preparation of special diets for diabetes, but further study is required to isolate, purify, characterize and identify the most active phytochemicals responsible for the anti-hyperglycemic effect of TOC and RKB.

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Conflict of Interest

The authors declared no potential conflicts of interest.

References

- Nair SS, Kavrekar V, Mishra A. 2013. *In vitro* studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. *Eur J Exp Biol* 3(1): 128-132.
- Yusoff NA, Yam MF, Beh HK, Niza K, Razak A, et al. 2015. Anti-diabetic and antioxidant activities of *Nypa fruticans* Wurmb. vinegar sample from Malaysia. *Asian Pac J Trop Med* 8(8): 595-605. <https://doi.org/10.1016/j.apjtm.2015.07.01>
- International Diabetes Federation. [<https://diabetesatlas.org/atlas/tenth-edition/>] Accessed on February on March 08, 2022.
- McMaken M, Shah S. 2017. A plant based-diet for prevention and treatment of type 2 diabetes. *J Geriatr Cardiol* 14(5): 342-354. <https://doi.org/10.11909/j.issn.1671-5411.2017.05.009>
- Adekola KA, Salleh AB, Zaidan UH, Azrina A, Chiavaro E, et al. 2017. Total phenolic content, antioxidative and antidiabetic properties of coconut (*Cocos nucifera* L.) testa and selected bean seed coats. *Ital J Food Sci* 29: 742-753. <https://doi.org/10.14674/IJFS-941>
- Romero-Arenas O, Damián Huato M, Rivera Tapia JA, Báez Simón A, Huerta Lara M, et al. 2013. The nutritional value of beans (*Phaseolus vulgaris* L.) and its importance for feeding rural communities in Puebla-Mexico. *Int Res J Bio Sci* 2(8): 59-65.
- DOA. 2016. Important nutritional information of popular grain legume varieties of Sri Lanka. Agriculture Publication Unit, Department of Agriculture, Peradeniya, Sri Lanka.
- Lijiao Kan, Shaoping Nie, Jielun Hu, Sunan Wang, Zhouya Bai, Junqiao Wang, Yaomin Zhou, Jun Jiang, Qin Zeng, Ke Song 2018. Comparative study on the chemical composition, anthocyanins, tocopherols and carotenoids of selected legumes. *Food Chem* 260: 317-326. <https://doi.org/10.1016/j.foodchem.2018.03.148>
- Marasinghe SSK, Marikkar JMN, Yalagama C, Wimalasiri S, Senviratne PRG, et al. 2019. Comparison of inter-varietal differences in chemical composition and nutritional properties of coconut testa flour. *J Natl Sci Found* 47: 349-356. <http://doi.org/10.4038/jnsfsr.v47i3.8699>
- Sangeetha A, Prasath GS, Subramanian S. 2014. Antihyperglycemic and antioxidant potentials of *Sesbania grandiflora* leaves studied in STZ induced experimental diabetic Rats. *Int J Pharma Sci Res* 5(6): 2266-2275. [https://doi.org/10.13040/IJPSR.0975-8232.5\(6\).2266-75](https://doi.org/10.13040/IJPSR.0975-8232.5(6).2266-75)
- Hiyoshi T, Fujiwara M, Yao Z. 2019. Postprandial hyperglycemia and postprandial hypertriglyceridemia in type 2 diabetes. *J Biomed Res* 33(1): 1-16. <https://doi.org/10.7555/JBR.31.20160164>
- Tanko AS, Marikkar JMN. 2021. Anti-hyperglycemic effect of bran extracts of two traditional rice varieties on Alloxan-induced diabetic rats. *Ind J Trad Knowl* 20(3): 707-715.
- Babes A, Fischer MJM, Filipovic M, Engel MA, Flonta M, et al. 2013. The anti-diabetic drug Glibenclamide is an agonist of the transient receptor potential ankyrin 1 (TRPA1) ion channel. *Eur J Pharm* 704(1-3): 15-22. <https://doi.org/10.1016/j.ejphar.2013.02.018>
- Gunarathna KMRU, Marikkar JMN, Mendis E, Yalagama C. 2022. Bioactivity studies of different solvent extracts of partially defatted coconut testa obtained from selected coconut cultivars. *J Agric Sci-S Lanka* 17: 171-184. <http://doi.org/10.4038/jas.v17i1.9618>
- Naveed M, Hejazi V, Abbas M, Kambh AA, Khan GJ, et al. 2018. Chlorogenic acid (CGA): A pharmacological review and call for further research. *Biomed Pharmacother* 97: 67-74. <https://doi.org/10.1016/j.biopha.2017.10.064>
- Kumar S, Kumar V, Rana M, Kumar D. 2012. Enzymes inhibitors from plants: an alternate approach to treat diabetes. *Pharmacognosy Communications* 2(2): 18-33.
- Ighodaro OM, Omole JO, Adejuwon AO, Odunaiya AA. 2012. Effects of Parinari polyandra seed extract on blood glucose level and biochemical indices in Wistar Rats. *Int J Diabetes Res* 1(4): 68-72.
- Ojo RJ, Segilola LI, Ogundele OM, Akintayo CO, Seriki S. 2013. Biochemical evaluation of lima beans (*Phaseolus lunatus*) in Alloxan-induced diabetic rats. *ARPN J Agric Bio Sci* 8(4): 302-309.
- Wang Y, Qiu X, Lv L, Wang C, Ye Z, Li S, Liu Q, Lou T, Liu X, et al. 2016. Correlation between serum lipid levels and measured glomerular filtration rate in Chinese patients with chronic kidney disease. *PLoS One* 11(10): e0163767. <https://doi.org/10.1371/journal.pone.0163767>
- Shin HS, Jung YS, Rim H. 2011. Relationship of serum bilirubin concentration to kidney function and 24-hour urine protein in Korean adults. *BMC Nephrol* 12: 29. <https://doi.org/10.1186/1471-2369-12-29>
- Zhu B, Wu X, Bi Y, Yang Y. 2017. Effect of bilirubin concentration on the risk of diabetic complications: A meta-analysis of epidemiologic studies. *Sci Rep* 7: 41681. <https://doi.org/10.1038/srep41681>
- Li J, Liu D, Liu Z. 2021. Serum total bilirubin and progression of chronic kidney disease and mortality: a systematic review and met-analysis. *Front Med (Lausanne)* 7: 549. <https://doi.org/10.3389/fmed.2020.00549>
- Gul A, Rahman MA. 2006. Comparison of blood protein levels between diabetic and non-diabetic patients with retinopathy. *J Coll Physicians Surg Pak* 16(6): 408-411.
- Nazki FA, Syeeda A, Mohammed S. 2017. Total protein, albumin and HBA1c in type 2 diabetes mellitus. *International Journal of Biochemistry* 3(3): 40-42.
- McGill MR. 2016. The past and present of serum amino transferases and the future of liver injury biomarkers. *EXCLI J* 15: 817-828. <https://doi.org/10.17179/excli2016-800>
- El-Demerdash F, Yousef MI, El-naga NIA. 2005. Biochemical study on the hypoglycemic effects of onion and garlic on Alloxan-induced diabetic rats. *Food Chem Toxicol* 43(1): 57-63. <https://doi.org/10.1016/j.fct.2004.08.012>