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Antidiabetic and Antioxidant Activity of *Rubus apetalus* Poir. and *Rubus steudneri* Schweinf. Leaf Extract on Alloxan Induced Diabetes Mellitus

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Abstract

Background and objective: Chronic hyperglycemia in diabetes determines the overproduction of free radicals, and evidence is increasing that these contribute to the development of diabetic complications. Aim of the present study was to evaluate the antidiabetic and antioxidant activity of *R. apelatus* Poir. and *R. steudneri* Schweinf leaf extract in alloxan induced diabetic rats.

Methods: The powdered materials of the *R. apelatus* Poir. and *R. steudneri* Schweinf were extracted by maceration process and the extract was used for the study. Single oral dose of 2000 mg/kg body weight of both the extracts were evaluated for acute toxicity. This study evaluated 150 and 300 mg/kg body weight of *R. apelatus* Poir. and *R. steudneri* Schweinf leaf extract for given orally for 21 days for its antidiabetic and antioxidant activity in alloxan induced diabetic rats.

Results: The results showed no toxicity in terms of general behavior change and mortality and LD50 was found to be more than 2000 mg/kg. Doses of 150 and 300 mg/kg body weight given orally for 21 days showed significant antihyperglycemic activity. Antihyperlipidemic and antiatherogenic activities as evidenced by significant decrease in total cholesterol, triglycerides, Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL) and Glycerol Free Triglyceride (GFT) levels coupled together with elevation of High Density Lipoprotein cholesterol (HDL-c) level and diminution of atherogenic index in diabetic rats. Furthermore, both the extracts significantly increases the activities and levels of Superoxide dismutase (SOD), Catalase (CAT), total thiols and Glutathione (GSH) and decreased the Lipid Peroxidation (MDA) showed the antioxidant activity.

Conclusion: Results of the present study suggests that both the extracts can control blood glucose and modulate the metabolism of glucose and blood lipid, leading to decreased outputs of lipid peroxidation and scavenge the free radicals in rats with diabetes mellitus.

Keywords: Diabetes mellitus; Lipid peroxidation; β-cells; Antioxidants

Abbreviations: LD50: Lethal Dose 50%; OECD: Economic Cooperation and Development Guideline; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein, GFT: Glycerol Free Triglyceride; HDL-c: High Density Lipoprotein Cholesterol; SOD: Superoxide Dismutase; CAT: Catalase; GSH: Glutathione; MDA: Malondialdehyde; ROS: Reactive Oxygen Species; RNS: Reactive Nitrogen Species; AI: Atherogenic Index

Introduction

Diabetes mellitus is a metabolic disorder disease, which is clinically diagnosed by a substantial increase in blood sugar levels as a result of the dysfunctional effect of pancreas that produce insufficient insulin from the β-cells [1]. Insufficient insulin secretion is also caused by disturbances in protein, carbohydrates and lipid metabolic processes [2]. Approximately 7% of the world population is currently suffering from Diabetes mellitus disease. The implication is that the epidemiologic incidence of diabetes mellitus is currently estimated at more than 500 million people worldwide. The expectation is that by the year 2045, almost 693 million people worldwide will have contracted diabetes mellitus with its direct healthcare-related costs expected to reach $1 trillion [3].

The oxidative stress process has been found to play a significant part in the overall pathogenesis of diabetes mellitus [4]. The oxidative stress process takes place when the free radical molecules react with chemical nutrients in the body (proteins, lipids, acids) with the resulting effect of generating Reactive Oxygen Species (ROS) and the Reactive Nitrogen Species (RNS) [5-7]. The stated lipid and protein oxidation results in a dysfunctional pancreatic β-cells with the ultimate adverse effect of producing insufficient insulin in the body [8] In addition, the nutrient oxidation process due to excess free radicals tends to inhibit the functional operations of antioxidants in the body [9,10]. The implication is that the oxidative stress process contributes significantly to the pathogenesis of diabetes mellitus due to the resulting imbalance between the oxidation process and the anti-oxidation process in the body [11,12]. The main complications resulting from the oxidative stress process in the body is the condition of hyperglycemia, which regenerates to diabetes mellitus [13]. In addition, the other complication of the oxidative stress process is the occurrence of other chronic diseases such as cardiovascular and hepatic diseases [5,14]. The production of free radicals and the resulting oxidative stress process is inhibited in the body by the functional operations of the antioxidant enzymes in the body [5,15].

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Rubus apetalus Poir. and *Rubus steudneri* Schweinf. belongs to the family Rosaceae. The leaves of the both the plants are common herbal medicines that are used in the treatment of diabetes mellitus and other associated disease complications. Specifically, the antioxidant effect of the Rubus species, *R. apelatus* Poir. and *R. steudneri* Schweinf has been demonstrated and confirmed in numerous laboratory studies. The antioxidant effect of both the plants has also been attributed to play a key role in the overall inhibition of the free radical molecules [9,11,16]. Preliminary phytochemical screening of the leaf extract of *R. apelatus* showed the presence of saponins, alkaloids, flavonoids, phenolics, tannins, phytosterols and triterpenoids [11,16] and *R. steudneri* contains phytochemicals such as saponins, alkaloids, flavonoids, and tannins [9,16]. The stated phytochemical compounds play a significant role in the inhibition of the free radicals and the oxidative stress process in the body due to their scavenging of hydroxyl radicals [9,11,16]. Keeping in view of all these facts about pathophysiological complications of diabetes and therapeutic efficacy of herbal medicines against diabetes, the following objectives have been drawn to study the efficacy of *R. apelatus* and *R. steudneri* leaf extract against alloxan induced diabetes model.

Methods

Collection and identification of plant

The *R. apetalus* and *R. studeneri* leaf material were collected from Nekemte, located in the East Wollega Zone of the Oromia Region, Ethiopia has a latitude and longitude of 9°5′N 36°33′E/9.083°N 36.550°E and an elevation of 2,123 meters above sea level. The plant *R. apetalus* (EBI No: 188; dated: 03-10-2015 collected by Abiyot Birhanu) and *R. studeneri* (EBI No: 1983; dated: 07-03-2002 collected by Kumlachew and Simon) were authenticated and confirmed by botanist based on its characteristics by comparing with herbarium deposited in Ethiopian Biodiversity Institute, Addis Ababa, Ethiopia.

Extraction

Maceration process was employed for extraction of both the powdered leaf material. The leaf powder (10 g) was soaked in double distilled water (100 ml) in a stoppered container and the container was left for 3 days at room temperature. The container was stirred frequently. After 3 days, the contents were filtered and the filtrate was subjected to evaporation (in a rotary evaporator) to get concentrated extract [9,11,17]. The concentrated extract was used for the evaluation of antidiabetic activity.

Animal care

Animals were kept for 1 week in our laboratory before the experiments for acclimatization to the laboratory conditions and fed with standard pellet diet and water *Ad libitum*. All rats were housed in polypropylene cages in a temperature (25°C ± 2°C) and humidity (60% ± 10%) controlled room submitted to a 12-dark/light cycle (artificial lights, 7 am-7 pm.) and air exhaustion cycle (15 min/h). All procedures were carried out in accordance with the conventional guidelines for experimentation with animals. Prior to all experiments, animals were fasted overnight but were allowed free access to water. Animal care and protocols were in accordance with and approved by the Institutional Animal Ethics Committee, Wollega University, Nekemte, Ethiopia.

Acute toxicity testing (limit test at 2000 mg/kg)

Swiss albino mice of either sex (25-40 g) were selected for the test. Acute toxicity study was carried out using acute toxic class limit test dose guidelines 425 of Organization for Economic Co-operation and Development (OECD). Twenty mice were randomly divided into four groups of five animals per sex. Acute toxicity of the *R. apetalus* and *R. studeneri* leaf extracts was carried out, using groups of five swiss albino mice per sex, by administering a dose of 2000 mg/kg body weight, and the control group was received normal saline. The toxicological effects were assessed based on mortality and behavioral changes occurred during 48 hours [18].

Induction and treatment of diabetes

The rats were injected with alloxan monohydrate in saline (0.9% NaCl) at a dose of 120 mg/kg b.w. intraperitoneally to induce diabetes in 8 h fasted male wistar rats weighing 180-200 g. After one hour of alloxan administration, the animals were given feed ad libitum. A 5% dextrose solution (10 g) was given in feeding bottle for a day to overcome the early hypoglycemic phase. After 72 h, animals with blood glucose levels higher than 220 mg/dL were considered diabetic and were included in the study [19].

Animals were divided into seven groups. Six groups were comprised of diabetic animals and one group of normal animals.

Group I: Normal control animal received 1 mL of distilled water.

Group II: Diabetic control received only 1 mL of distilled water orally.

Group III: Diabetic animal received *Rubus steudneri* Schweinf. leaf extract (150 mg/kg b.w.) orally.

Group IV: Diabetic animal received *Rubus steudneri* Schweinf. leaf extract (300 mg/kg b.w.) orally.

Group V: Diabetic animal received *Rubus apetalus* Poir. leaf extract (150 mg/kg b.w.) orally.

Group VI: Diabetic animal received *Rubus apetalus* Poir. leaf extract (300 mg/kg b.w.) orally.

Group VII: Diabetic animal received reference drug glibenclamide (5 mg/kg b.w.) orally.

Animals of different groups were given treatment according to their respective group for 28 days. After 28 days, blood was collected for biochemical analysis and animals were dissected. The organs (pancreas, liver, and kidney), were collected for histological examination, tissue processing and staining was performed as per the method describe by Dunn [20].

Preparation of liver homogenate

The whole liver was perfused *in situ* with ice cold saline, dissected out, blotted dry and immediately weighed. A 10% w/v liver homogenate was prepared in ice cold 1.15% KCl using Teflon glass homogenizer (Yamato LSG LH-21, Japan). The homogenate was centrifuged at 10,000 rpm for 10 min at 4°C and the supernatant was used for the estimation of proteins and lipid peroxidation. The supernatant was again centrifuged at 20,000 rpm for 1 hour at 4°C. The supernatant obtained was used for the estimation of Glutathione (GSH), total thiols, Superoxide dismutase (SOD) and Catalase (CAT) activity.

Estimation of lipid peroxidation and antioxidant enzymes

Lipid peroxidation estimation was performed as per the method of Niehaus and Samuelsson [21]. Protein content of the liver homogenate was determined by the method of Lowry et al. [22] using bovine serum albumin as standard. GSH content was measured by the method of

Moran et al. [23]. The total thiols estimation was performed as per the method described by Sedlak and Lindsy [24]. The activity of superoxide dismutase was performed by the method of Misra and Fridovich [25] and the catalase activity was determined spectrophotometrically according to the protocol of Claiborne [26].

Statistical analysis

All the data were expressed as mean ± SEM. The significance of difference in means between control and treated animals for different parameters was determined by using One way Analysis of Variance (ANOVA) followed by multiple comparison Tukey's test. Significance of differences between groups was evaluated with Students t-test. A p-value of <0.05 was considered statistically significant.

Results

Acute toxicity testing (limit test at 2000 mg/kg b.w.)

The results of the acute toxicological study showed that the administration of both the leaf extracts by oral route at does up to 2000 mg/kg did not produce any mortality in experimental animals. Animals were observed for behavioural signs of toxicity include motor activity, tremors, clonic convulsions, tonic convulsions, straub's phenomenon, catatonia, sedation, diarrhea, lacrimation, salivation, writhing and irritation and there was no such behavioural signs of toxicity were recorded during 48 hours, which was the no observed adverse effect level (NOAEL) [27]. LD50 of both the extracts were found to be more than 2,000 mg/kg/p.o. Body weight, water and food intake were not affected during 14 days of observation.

Effect of *R. apetalus* and *R. studeneri* leaf extracts on body weight

Table 1 represents the body weight of alloxan induced diabetic rats treated with *R. studeneri* (Group III (150 mg/kg b.w) and Group IV (300 mg/kg b.w)), *R. apetalus* (Group V (150 mg/kg b.w) and Group VI (300 mg/kg b.w)), 5 mg/kg b.w of glibenclamide (Group VI) the diabetic positive control (Group II), and of the non diabetic control (Group I). Normal group (Group I) of rats remained fairly stable from 1st day to the 28th day during the period of experimentation. On the other hand, the body weight of the control sample group, experimental group and the standard group declined in the first week but increased significantly during the next 3 weeks (Table 1). The results indicated that decline in body weight of the alloxan-induced diabetic rats is not significant up to the 7th day. However, the increase in body weight during the following three weeks is statistically significant ($p<0.001$) upon oral administration of different doses of *R. apetalus* and *R. studeneri* leaf extracts and reference drug glibenclamide for 21 days.

Effect of *R. apetalus* and *R. studeneri* leaf extracts on blood glucose

Table 2 depicts the antihyperglycemic effect of *R. apetalus* and *R. studeneri* leaf extracts on fasting blood glucose levels of diabetic rats showed. Administration of alloxan (120 mg/kg b.w., i.p.) led to 1.5-2 fold elevation of fasting blood glucose levels, which was maintained over a period of 3 weeks. The blood glucose was significantly ($p<0.001$) elevated in diabetic rats as compared to normal rats. Oral administration of R. apetalus (Group III and IV) and *R. steudneri* (Group V and VI) leaf extracts for 21 days at 150 and 300 mg/kg b.w significantly ($p<0.01$) lowered the blood glucose as compared to untreated diabetic rats (Group II). Administration of the reference antidiabetic drug glibenclamide significantly ($p<0.001$) lowered the fasting blood glucose level when compared with diabetic control rats.

Effect of *R. apelatus* and *R. steudneri* leaf extracts on lipid profile

Administration of alloxan significantly increased the levels of triglyceride ($p<0.001$), total cholesterol ($p<0.001$), LDL ($p<0.01$), VLDL ($p<0.01$), GFT ($p<0.01$) and significantly ($p<0.001$) lowered the levels of HDL, when compared with normal control (Group I) and the data were represented in the Table 3. Oral administration of *R. apelatus* and *R. steudneri* leaf extracts for 21 days significantly decreased the levels of triglyceride ($p<0.001$), total cholesterol ($p<0.001$), LDL ($p<0.001$), VLDL ($p<0.001$), GFT ($p<0.001$) and significantly ($p<0.01$) lowered the levels of HDL, when compared with normal control (Group I). The highest dose (300 mg/kg b.w) showed highest activity, when compared to the lowest dose (150 mg/kg b.w). The results were compared with the reference drug glibenclamide and showed a significant ($p<0.001$) activity. A increased atherogenic index (AI) was observed in diabetic rats compared to normal control. However, remarkable decrease ($p<0.01$) was found in atherogenic index in 150 and 300 mg/kg of *R. apelatus* and *R. steudneri* leaf extracts and glibenclamide for 21 days treated animals as compared with diabetic group (Group II).

Effect of leaf extracts on lipid peroxidation and antioxidant enzymes

The activities of MDA and enzymatic and non-enzymatic antioxidants [Glutathione, Total Thiols, superoxide dismutase (SOD) and catalase (CAT)], in liver of normal and experimental animals in each group was shown in Table 4. Administration of both leaf extracts (*R. apelatus* and *R. steudneri*) and the standard antidiabetic drug enhanced the activity of SOD, GSH, Total thiols and catalase compared to the control group (Group II). The oral administration of leaf extracts and glibenclamide for 21 days significantly reduced the lipid peroxidation compared to diabetic control (Group II). The highest dose (300 mg/kg b.w) showed highest activity when compared with lower dose (150 mg/kg b.w).

Histopathological analysis

Figure 1 presents a summary of the photomicrographs related to histological sections liver, kidney and pancreas for the normal, control and diabetic-treated rats treated with *R. apelatus*, *R. steudneri* and glibenclamide.

Histopathology of liver

The photomicrographs presented in Figure 1 indicate that the liver section of normal rats (I) had normal histological structural appearance with central veins, hepatocytes, nucleus, and sinusoid tissues. On the other hand, the liver sections of non-treated control group of alloxan-induced diabetic rats (II) indicate presence of damaged and distended portal veins as well as inflammation in the leucocytes and fibrosis. Finally, the liver sections of rats treated with the leaf extracts of *R. apelatus* and *R. steudneri* and glibenclamide (III, IV, V, VI and VIII) show normal hepatocytes, central vein and nucleus histological structures with only mild dilation in sinusoid tissues in group III and V.

Histopathology of kidney

The photomicrographs presented in Figure 1 indicate that the histological structure of kidney tissues in normal rats (I) has no inflammatory structural alterations with the convoluted tubules and glomerulus being normal. However, the kidney tissues of the non-treated alloxan-induced rats (II) show inflammation of the convoluted

Group	0th Day	3rd Day	7th Day	14th Day	21st Day	28th Day
I	288.1 ± 7.440	295.3 ± 2.504	244.3 ± 18.53	292.9 ± 5.10	285.3 ± 5.04	285.3 ± 5.04
II	259.4 ± 21.09	113.7 ± 36.87^{a}	127.1 ± 17.69^{c}	159.6 ± 34.93^{a}	198.9 ± 22.61^{a}	203.8 ± 13.24^{a}
III	267.3 ± 7.849	183.1 ± 51.65^{ns}	237.9 ± 41.04*	268.7 ± 4.61*	276.9 ± 8.85***	294.3 ± 10.12***
IV	272.9 ± 14.17	220.0 ± 19.27^{ns}	234.14 ± 37.64^{ns}	282.1 ± 7.99***	286.7 ± 4.88***	292.5 ± 6.55***
V	296.1 ± 2.251	237.9 ± 27.21^{ns}	266.4 ± 16.01**	279.6 ± 6.21***	292.0 ± 2.42***	299.1 ± 10.79***
VI	273.6 ± 7.422	271.7 ± 14.58**	285.3 ± 5.506***	284.4 ± 3.96***	286.4 ± 5.62***	294.9 ± 8.23***
VII	257.24 ± 8.61	209.4 ± 16.03 ^{ns}	224.9 ± 12.09^{ns}	261.3 ± 15.46**	283.6 ± 17.59***	287.7 ± 13.17***
All the values are mean ± SEM, One way analysis of Variance (ANOVA) followed by Tukey's multiple comparision test: ^{a}p<0.05,^{b}p<0.01,^{c}p<0.001 as comparison to normal group (Group I); *p<0.05,**p<0.01, ***p<0.001 as comparison to Diabetic control group (Group II). ns: non-significant.						

Table 1: Body weight (in grams) changes in normal and experimental animals in each group at the 0th, 3rd, 7th, 14th, 21st and 28th day.

Group	Blood glucose level (in mg/Dl)					
	0 day	3rdday	7thday	14thday	21stday	28thday
I	111.5 ± 6.44	108.8 ± 4.678	127.9 ± 18.92	109.4 ± 3.449	111.8 ± 4.290	99.42 ± 7.32
II	105.2 ± 4.95	384.8 ± 42.64^{b}	396.0 ± 29.92^{a}	366.8 ± 38.05^{b}	309.5 ± 31.37^{a}	345.7 ± 37.29^{b}
III	114.9 ± 4.48	376.5 ± 16.95	317.7 ± 18.70	300.0 ± 11.79	287.6 ± 5.92	219.7 ± 14.73*
IV	118.8 ± 7.31	352.9 ± 38.16	306.6 ± 42.68	274.2 ± 28.04	221.5 ± 26.83*	187.3 ± 18.95**
V	116.5 ± 5.82	387.5 ± 13.05	324.7 ± 10.17	298.0 ± 9.739	267.6 ± 5.92*	200.7 ± 12.37**
VI	112.8 ± 6.24	362.5 ± 29.85	305.6 ± 16.35	271.2 ± 17.04	221.5 ± 26.8*	179.3 ± 08.19**
VII	93.82 ± 6.95	302.5 ± 15.17	291.4 ± 24.94	195.5 ± 39.92**	158.8 ± 16.15***	144.1 ± 5.42***
All the values are mean ± SEM, One way analysis of Variance (ANOVA) followed by Tukey's multiple comparision test: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 as comparison to normal group (Group I); *p<0.05, **p<0.01, ***p<0.001 as comparison to Diabetic control group (Group II). ns: non-significant.						

Table 2: Changes in fasting blood glucose level (mg/dL) of normal and experimental animals in each group after the administration of *R. apelatus* and *R. steudneri* leaf extracts for 21 days.

Group	Triglyceride (mg/dl)	TC (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	GFT (mg/dl)	AI (mg/dl)
I	66.5 ± 1	162.5 ± 0.98	37.5 ± 0.7	111.6 ± 1.5	13.3 ± 0.2	56.4 ± 0.9	2.89 ± 0.1
II	99.2 ± 0.8^{c}	194.4 ± 1^{c}	27.6 ± 1.3^{c}	126.9 ± 1.7^{b}	19.8 ± 0.1^{b}	89.2 ± 0.8^{b}	3.09 ± 0.1^{b}
III	65.6 ± 1.5***	167.6 ± 1.2***	48.4 ± 2.5*	107 ± 2.7***	13.1 ± 0.3***	55.6 ± 1.5**	2.58 ± 0.1**
IV	52.1 ± 0.9***	156.9 ± 1.7***	52.7 ± 1.4**	93.7 ± 1.3***	10.5 ± 0.1***	42.1 ± 0.9***	1.98 ± 0.2**
V	40.5 ± 0.8***	145.7 ± 1.2***	61.3 ± 0.6***	76.1 ± 1.6***	8.10 ± 0.1***	30.5 ± 0.8***	1.37 ± 0.3**
VI	37.5 ± 0.4***	17.7 ± 1.1***	59.3 ± 0.3***	67.1 ± 1.6***	7.9 ± 0.1***	34.5 ± 0.4***	1.28 ± 0.2**
VII	53.3 ± 1***	152.8 ± 1.3***	61 ± 1.2***	81.4 ± 2***	10.6 ± 0.2***	43.3 ± 1***	1.51 ± 0.1**
All the values are mean ± SEM, One way analysis of Variance (ANOVA) followed by Tukey's multiple comparision test: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 as comparison to normal group (Group I); *p<0.05, **p<0.01, ***p<0.001 as comparison to Diabetic control group (Group II). ns: non-significant, HDL- High Density Lipoprotein, TC-Total Cholesterol, LDL- Low Density Lipoprotein, VLDL- Very Low Density Lipoprotein, GFT- Glycerol Free Triglyceride, AI- Atherogenic Index.							

Table 3: Effect of *R. apelatus* and *R. steudneri* leaf extracts administration for 21 days on lipid profile and anti-atherogenic index in alloxan induced diabetic rats.

Group	Lipid peroxidation (nm/mg of protein)	GSH (nm/mg of protein)	Total thiols (µm/mg of protein)	SOD (U/mg of protein)	Catalase (U/mg of protein)
I	95.65 ± 20.05	58.82 ± 16.60	146.7 ± 47.18	374.0 ± 35.93	0.09481 ± 0.02
II	160.7 ± 77.86^{a}	24.61 ± 6.860^{a}	31.53 ± 10.86	305.5 ± 132.1	0.05345 ± 0.06
III	124.4 ± 68.97	25.93 ± 4.338	42.54 ± 28.45	385.6 ± 889.4*	0.06563 ± 0.02
IV	100.2 ± 23.33**	76.33 ± 21.70**	108.7 ± 29.40**	366.1 ± 92.63*	0.07437 ± 0.03
V	90.68 ± 41.53**	55.82 ± 22.68**	196.3 ± 56.70***	478.5 ± 103.4**	0.08756 ± 0.03
VI	89.2 ± 15.31**	78.33 ± 22.70**	215.7 ± 14.40**	362.1 ± 28.63*	0.06127 ± 0.02
VII	88.68 ± 21.53**	38.82 ± 26.18**	199.3 ± 14.70***	324.5 ± 11.4	0.07416 ± 0.01
All the values are mean ± SEM, One way analysis of Variance (ANOVA) followed by Tukey's multiple comparision test: ^{a}p<0.05, ^{b}p<0.01, ^{c}p<0.001 as comparison to normal group (Group I); *p<0.05, **p<0.01, ***p<0.001 as comparison to Diabetic control group (Group II). ns: non-significant, GSH: Glutathione and SOD: Superoxide Dismutase.					

Table 4: Effect of administration *R. apelatus* and *R. steudneri* leaf extracts for 21 days on the levels of MDA and activities and levels of non-enzymatic and enzymatic antioxidants.

tubules as well as contraction of the glomerulus. Finally, the liver sections of rats treated with the leaf extracts of *R. apelatus* and *R. steudneri* and glibenclamide (III, IV, V, VI, and VIII) depict normal glomerulus and convoluted tubules structures with no inflammation.

Histopathology of pancreas

The photomicrographs presented in Figure 1 indicate that the pancreas of normal rats (I) had normal structural islets of langerhans,

which seem to be partially stained compared to the acinar cells. On the other hand, the histological structure of the pancreas of non-treated alloxan-induced diabetic rats (II) indicates presence of acinar cells, which are swollen with tiny vacuoles being evident in the stated cells. In addition, in group II rats, flattened epithelium was evident along interlobular ducts. Finally, the pancreas sections of rats treated with the leaf extracts of *R. apelatus* and *R. steudneri* and glibenclamide (III, IV, V, VI, and VIII), show that the acinar cells look normal while there seems to be a clear border between the exocrine and the endocrine pancreatic tissues.

Discussion

Diabetes mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases. Currently, available therapeutic options for non-insulin dependent diabetes mellitus such as dietary modification, oral hypoglycemics and insulin have limitations of their own. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future. There are no reports available on the antidiabetic and antioxidant activity of *R. apelatus* and *R. steudneri*. In this study, we made an attempt to study the antidiabetic and antioxidant activity of *R. apelatus* and *R. steudneri* leaf extracts on alloxan induced diabetic rats.

The body weight in alloxan-induced diabetic rats decreased significantly, while the body weight of the control, experimental and standard group increased significantly during the 21 days of drug administration. The study of Panda et al. [1] found that, the body weight of diabetic rats increased substantially due to administration of Swietenia mahagoni leaf extracts in diabetic rats. The main justification for increased body weight in both alloxan-induced and streptozotocin-induced rats is due to the antioxidant effect of the respective leaf extracts, which may be due to the reduced lipid peroxidation and increased HDL level [1,16,28].

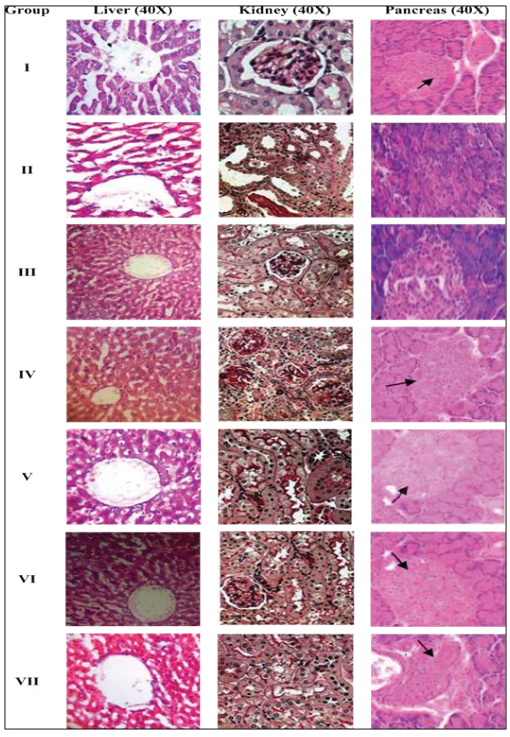

Figure 1: Photomicrographs (40X) of the histological sections of liver, kidney and pancreas.

The oral administration of both the leaf extracts (*R. apelatus* and *R. steudneri*) contributed to significant decline in blood glucose levels. However, the results showed that use of glibenclamide antidiabetic drug resulted in the highest significant decline in blood glucose levels compared to administration of the leaf extracts. In contrast, the results based on the study of Kumar and Saleem [28] indicate that use of *Asystasia gangetica* and *Morus indica* in alloxan-induced diabetic albino rats resulted in significant decline in blood glucose levels compared to administration of the standard antidiabetic drug. The main justification for reduced levels of blood glucose in alloxan-induced rats is that the stated herbal leaf extracts stimulate the production of increased amount of hepatic glycogen in liver, which inhibits the process of gluconeogenesis in the body [5,28]. Therefore, the antioxidant effect of most herbal leaf extracts is mainly based on their inhibiting effect on gluconeogenesis due to production of hepatic glycogen in the liver [29,30].

The study reveals that use of leaf extracts (*R. apelatus* and *R. steudneri*) and glibenclamide resulted in low levels of triglycerides, TC, LDL, VLDL, GFT and AI. However, increased administration of the stated leaf extracts and the standard antidiabetic drug enhanced the quantity of high density cholesterol (HDL), which is good for the body. The results on the effect of samples on the biochemical characteristics of the alloxan-induced rats is similar to findings of the study by Tadesse et al. [16] who observe that the reduction in lipids and bad cholesterols in diabetic rats is facilitated by the antioxidant effect of the herbal leaf extracts. In addition, Raghavendra et al. [9,11] also argue that the radical scavenging attributes of the *R. steudneri* fruits tend to inhibit the lipid radicals, which contribute to lipid peroxidation process [5,9,31,32].

The administration of *R. apelatus* and *R. steudneri* as well as glibenclamide inhibited the process of lipid peroxidation in the body. In addition, the results depict that lipid peroxidation was greatly inhibited with administration of higher quantity of the leaf extracts. On the other hand, the administration of *R. apelatus* and *R. steudneri* resulted in increased production of antioxidant enzymes (GSH, total thiols, SOD and catalase). However, production of SOD and catalase antioxidant enzymes was highest with use of glibenclamide drug compared to administration of the stated leaf extracts. The results seem to be consistent with the study of Kumar et al. [28] and Raghavendra et al. [9,11], who observe that increased production of GSH and total thiols with administration of herbal leaf extracts is due to the effect of reduced lipid peroxidation on alloxan-induced rats [5,9,11,28]. On the other hand, the antioxidant effect of the herbal leaf extracts is due to the fact that increased levels of SOD and catalase antioxidants facilitate the conversion of free radicals (superoxide) into hydrogen peroxide and oxygen molecules [33].

Finally, the results based on the pathophysiological structure of the normal, control and experimental groups indicate that the use of the leaf extracts and the antidiabetic drug (glibenclamide) resulted in less severe structural changes in tissues compared to the non-treated diabetic rats. The antioxidant and scavenging attributes of the leaf extracts of *R. apelatus* and *R. steudneri* seem to have inhibited the adverse actions of the free radicals, which prevented severe structural changes in the kidney, liver and pancreas of the treated diabetic rats in the experiments [34,35]. In addition, the use of the stated leaf extracts also seems to have enhanced the functions of the β-cells in the pancreas of the diabetic treated rats in the experiment [34].

Conclusions

This paper examines the antidiabetic and antioxidant effects of the *R. apelatus* and *R. steudneri* leaf extracts in alloxan-induced diabetic rats. Administration of leaf extracts resulted in a significant increase in body weight among the experimental group. However, the analysis based on blood glucose level indicates that the administration of *R. apelatus* and *R. steudneri* as well as the use of glibenclamide drug resulted in a significantly decreased glucose level from the 7th day to the 28th day. On the other hand, the results depict that use of the leaf extracts reduced the level of triglycerides and bad cholesterol, while at the same time increased the quantity of HDL in alloxan-induced diabetic rats. Furthermore, the administration of *R. apelatus* and *R. steudneri* leaf extracts and the standard diabetic drug significantly inhibited the lipid peroxidation, while increased the activities and levels of GSH, total thiols, SOD, and catalase antioxidant enzymes. Thus a *R. apelatus* and *R. steudneri* leaf extracts with these multiple advantageous properties viz., antidiabetic, antihyperlipidemic, antiatherogenic and antioxidant properties without causing hypoglycemia would be of greater therapeutic benefit in the management of diabetes mellitus associated with abnormalities in lipid profiles and merits further detailed investigation to find out its mechanism of action and to establish its therapeutic potential in the treatment of diabetes and diabetic complications.

Competing Interests

None declared.

Authors' Contributions

Hallur Lakshmanashetty Raghavendra designed and outlined the original project. Raghavendra HL, Upashe SP and Floriano JF analyzed and interpreted, reviewed the data and wrote the article. All authors critically revised the drat paper and have approved the final manuscript.

References

1. Panda SP, Haldar PK, Bera S (2010) Antidiabetic and antioxidant activity of Swietenia Mahagoni in streptozotocin-induced diabetic rats. J Pharmac Biol 48: 974-979.
2. Rajendiran D, Packirisamy S, Gunasekaran KA (2018) Review on role of antioxidants in diabetes. Asian J Pharm Clin Res 11: 48-53.
3. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, et al. (2018). IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract 138: 271-281.
4. Ganapaty S, Chandrashekar VM, Chitme HR, Narsu LM (2007) Free radical scavenging activity of gossypin and nevadensis: An in-vitro evaluation. Indian J Pharmacol 39: 281-283.
5. Tauchen J, Doskocil I, Caffi C, Lulekal E, Marsik P, et al. (2015) In vitro antioxidant and anti-proliferative activity of Ethiopian medicinal plant extracts. Ind Crops Prod 74: 671-679.
6. Jia W, Gao W, Tang L (2003) Antidiabetic herbal drugs officially approved in China. Phytother Res 17: 1127-1134.
7. Tiwari P, Kumar B, Kaur M, Kaur G, Kauret H (2011) Phytochemical screening and reaction: A review. Int Pharm Sci 1: 98-106.
8. Jiang X, Meng Y, Liang Y, Xiao Z (2015) Evaluation of the antioxidant and antibacterial activities of various solvent extracts from Passiflora wilsonii Hemsl. Biotechnol 14: 129-135.
9. Raghavendra HL, Kekuda PTR (2018) Preliminary phytochemical analysis, antiradical and lipid peroxidation inhibitory activity of Rubus Steudneri Schweinf. (Rosaceae). Int J Green Pharm 12: 49-53.
10. Nimse SB, Pal D (2015) Free radicals, natural antioxidants and their reaction mechanisms. RSC Adv 5: 27986-28006.
11. Raghavendra HL, Prashith KTR, Chetan DM (2018) Phytochemical analysis and in vitro antioxidant activities of Rubus apetalus Poir. (Rosaceae). Pharmacologyonline 1: 187-195.
12. Yussuf AZ, Zakir A, Shemau Z, Abdullahi M, Halima SA (2014) Phytochemical analysis of the methanol leave extracts of Paullinia pinnata Linn. J Pharmacognosy Phytother 6: 10-16.
13. Kekuda PTR, Raghavendra HL, Shilpa M, Pushpavathi D, Petkar T, et al. (2017) Antimicrobial, antiradical and insecticidal activity of Gardenia gummifera L.F. (Rubiaceae). Int J Pharm Pharm Sci 9: 265-272.
14. Lulekal E, Rondevaldova J, Bernaskova E (2014) Antimicrobial activity of traditional medicinal plants from Ankober district, north Shewa zone, Amhara region, Ethiopia. Pharm Biol 52: 614-620.
15. Nandhakumar E, Indumathi P (2013) In vitro antioxidant activities of methanol and aqueous extract of Annona squamosa (L.) fruit palp. J Acupunct Meridian Stud 6: 142-148.
16. Tadesse S, Asres K, Veeresham C (2007) Antioxidant activities of three Rubus species growing in Ethiopia. Ethiopian Pharma J 25: 103-110.
17. Yeo YL, Chia YY, Lee CH, Sow HS, Yap WS (2014) Effectiveness of maceration periods with different extraction solvents on in-vitro antimicrobial activity from fruit of Momordica charantia L. J Appl Pharm Sci 4: 16-23.
18. Guideline OT (2012) 425. 2001. Guidelines for testing of chemicals. Guidelines 425, acute oral toxicity-up-and-down procedure.
19. Jarald E, Joshi SB, Jain DC (2009) Biochemical study on the hypoglycaemic effects of extract and fraction of Acacia catechu willd in alloxan-induced diabetic rats. Int J Diabetes Metab 17: 63-69.
20. Dunn WL (1974) Hand book of histopathological and histochemical techniques. Third Edition, Redwood, Burn, Ltd, Trowbridge and Esher, London.
21. Niehaus WG, Samuelsson B (1968) Formulation of malonaldehyde from phospholipids arachidonate during microsomal lipid peroxidation. Eur J Biochem 17: 126-130.
22. Lowry OH, Rosebrough NJ, Faro AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193: 265-75.
23. Moran MS, Difieree JW, Mannerwik KB (1979) Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochim Biophys Acta 582: 67-78.
24. Sedlak J, Lindsy R (1968) Estimation of total protein bound and non-protein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25: 192-205.
25. Misra HP, Fridovich I (1972) The role of superoxide anion in the autooxidation of epinephrine and a simple assay for suproxide dismutase. J Biol Chem 247: 3170-3175.
26. Claiborne A (1985) CRC handbook of methods for oxygen radical research. Catalase activity pp: 283-284.
27. Alexeeff GV, Broadwin R, Liaw J, Dawson SV (2002) Characterization of the LOAEL-to-NOAEL uncertainty factor for mild adverse effects from acute inhalation exposures. Regul Toxicol Pharmacol 36: 96-105.
28. Kumar RP, Saleem TSM (2010) Potential antidiabetic and antioxidant activities of Morus indica and Asystasia gangetica in alloxan-induced diabetic mellitus. J Exp Pharmacol 2: 29-36.
29. Singh PP, Mahadi F, Roy A, Sharma P (2009) Reactive nitrogen species and antioxidants in etiopathogenesis of diabetes mellitus Type 2. Indian J Clin Biochem 24: 324-342.
30. Xu Y, Burton S, Kim C, Sismour E (2015) Phenolic compounds, antioxidants, and antibacterial properties of pornace extracts from four Virginia-grown grape varieties. Food Sci Nutr 4: 125-133.
31. Badmus JA, Adedosu TO, Fatoki JO, Adegbite VA, Adaramoye OA, et al. (2011) Lipid peroxidation inhibition and antiradical activities of some leaf fractions of Mangifera indica. Acta Pol Pharm 68: 23-29.
32. Sowndhararajan K, Kang SC (2013) Free radical scavenging activity from different extracts of leaves from Bauhinia vahlii Wight & Arn. Saudi J Biol Sci 20: 319-325.
33. Rahul S, Sanjay K, Abhay KS (2006) Antidiabetic potential of Butea monosperma in rats. Fitoterapia 76: 86-90.
34. Nurdiani S, Goh YM, Ahmad H, Dom SM, Azmi NS, et al. (2017) Changes in pancreatic histology, insulin secretion and oxidative status in diabetic rats following treatment with Ficus deltoidea and vitexin. BMC Complement Altern Med 17: 290-299.
35. Aboonabi A, Rahmat A, Othman F (2014) Effect of Pomegranate on histopathology of liver and kidney on generated oxidative stress diabetic induced rats. J Cytol Histol 6: 294.