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Original Article

Antioxidants Activity Assessment and Utilization of Banana Peels to Attenuate the Diabetes Mellitus

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ABSTRACT

Diabetes is the primary metabolic disorder listed among the top 10 death-causing diseases. The complete cure of diabetes is impossible, but the prevention and maintenance of glucose levels can reduce the diabetes severity. **Objectives:** To utilize the banana peel extracts to evaluate their antioxidant attributes and capability to attenuate diabetes. **Methods:** The antioxidant properties were assessed by measuring the DPPH, total phenolic contents (TPC), and total flavonoid contents (TFC) in ethanol, methanol, and acetone solutions. Moreover, the renal functional tests (Serum creatinine, serum urea, and BUN) and liver function tests (ALT, ASP, Serum Albumin, and total proteins) were also conducted during the 21 days experimental study in diabetes-induced (via Streptozotocin: 350 mg/kg) male Albino Wister rats. **Results:** The results indicated that the DPPH, TPC, and TFC contents were higher in methanol solution, i.e., 74.20±0.98%, 54.78±0.69mg GAE/g, and 39.48±0.37mg GAE/g respectively. Moreover, the results indicated that the unripe, ripe, and overripe significantly reduced liver and renal function parameters in diabetic rats. **Conclusions:** Banana peels have prominent potential to prevent diabetes-linked variables due to their higher antioxidant activity.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder mainly due to hyperglycemia. In this disease, the body fails to produce or respond to average insulin concentrations, leading to high blood glucose levels. Globally, its prevalence is estimated at 536.6 million people (10.5%) in 2021 among 20-79 years older people and is estimated to rise to 783.2 million (12.2%) in 2045. Its prevalence was equal in males and females but highest in people aged 75-79 [1]. It was listed among the ten top death reasons [2]. DM plays a significant role in changing protein, carbohydrates, and lipids [3]. Diabetes

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mellitus is divided into diabetes Type 1 (insulin-dependent) and diabetes type 2 (non-insulin-dependent). Both types collectively affect more than 346 million people, of which diabetes type accounts for 90% of patients. Different therapies are under consideration to prevent and treat diabetes mellitus. Fruits among the natural resources show significant potential to cure various diseases. These are diversified in nature among the plant food groups. Fruits contain multiple nutrients, i.e., carotenoids, polyphenols, and bioactive compounds, in preventing and reducing obesity, diabetes, and different cardiovascular disorders. Plant minerals, vitamins, and phytochemicals have a significant role against inflammatory disorders, oxidation activities, and protective roles against various disorders in the body [4, 5].Banana (belongs to the Musaceae family) is a most ancient and vital nutritional crop with evidence of its cultivation back to 4000BCE in New Guinea [6]. The banana peel contains higher amounts of nutrientssuch as cellulose (7.6-9.5 %), hemicelluloses (6.40-9.50 %), pectin (10-21 %), and lignin (6-12 %) and various polyphenols (from 0.9-3 g/100g) that can be utilized in the management of multiple disorders, i.e., hypoglycemic effects and antidiabetic properties [7]. The banana peelscontain higher amounts of potassium, sodium, calcium, manganese, copper, iron, zirconium, rubidium, niobium, strontium, and bromine [8]. The peels of green bananas have been reported to have significant potential to stimulate glucose utilization, insulin production, and hypoglycemic effect. The hypoglycemic effect is directly linked to higher sodium (Na) and Potassium (K) concentrations. Banana peel fibers help lower fasting blood glucose levels and increase hepatic glycogenosis [9]. Banana peels contain higher phenolic compounds than banana pulp and are considered a cheaper natural source of antioxidants [10]. The current study evaluated the antioxidant potential of banana peel at its various stages. Diabetes was induced in the rats by injecting Streptozotocin (STZ) rats, and the antidiabetic potential of banana peel powder was evaluated in hyperglycemic rats.

METHODS

The bananas were purchased from the local market in Lahore. Bananas were peeled, dirt particles removed from the peels, and the peels were further peeled. Peels were soaked in 0.5% citric acid-containing water for 10 min. Then, the peels were steamed to make them soft in a covered container at 85ºC for 10 min. After that, water and banana peels were mixed in the ratio (2:1), and flakes of banana peels were dried through a drum drier to achieve the <1 mm particle size. The approval was taken from the Department of Diet and Nutrition and Medical and Health Research Ethics Committee for efficacy study, and adult Albino Wister male rats (Rattus norvegicus) with weights ranging from 150-200 g were selected. The rats were acclimatized in cages at room temperature (22 °C) in 12 hours of light-dark cycles. Then rats were fed on a standard diet and tap water. The rats were divided into five groups named I, II, III, IV, and V. The diabetes was induced in four out of five groups. The rats were kept in fasting condition for 24 hours to maintain normal body conditions of rats before injecting Streptozotocin (STZ) (350 mg/kg) prepared in 0.5mLof saline solution via the peritoneal cavity. The blood glucose was assessed through the glucometer (On-Call Glucometer, EZ-II Version) after three days of diabetes induction. The glucose levels of hyperglycemic or diabetic rats were maintained at equal to or greater than 200 mg/dL throughout the study. The rats were given the standard basal diet and aqueous plant extract (250 mg/kg). The diet plan of the experimental rats consisted of five group's i.e., basal diet only (Normal control group), Basal diet only (Diabetic control group), basal diet + ripe banana peel (250 mg/kg Body weight), basal diet + unripe banana peel (250 mg/kg body weight), basal diet + over ripe banana peel (250 mg/kg body weight). The diet was given to the rats according to the diet mentioned above plan for three weeks. After 1 hour of the last meal, rats were put on a horizontal rod (19.69 inches) on the table surface, 1cm from the tail's tip (Tail Suspension Test). This test was used to measure the total immobility length, and immobility was recorded for 6 minutes via camera [11].The ketamine 70 mg/kg BW was injected into each rat's right or left thigh. When rats become unconscious, the blood was collected from the retro-orbital sinus after 72 hours for a diabetes test. The glucose was measured via Accu-Check Go glucometer (Certeza Glucometer, GL-110 Version) at the 7th, 14th, and 21st days to check for diabetes. The serum samples were analyzed via spectrophotometry. The blood chemistry of the rats was analyzed through the diagnostic kits and chemical analyzer using developed methods on blood samples. The liver and renal functional tests i.e., Creatinine (mg/dL) and Urea (mg/dL) test and some liver enzymes i.e., Bilirubin (g/dL), Albumin (g/dL), Total proteins (g/dL), Gamma-glutamyl transferase (GGT), Alkaline phosphatase (ALP), Aspartate transferase (AST) and Alanine aminotransferase [12]. The results were presented in mean ± SD. The one-way ANOVA was applied to analyze the data among various parameters such as body weight, blood glucose levels, and liver and renal functions by using SPSS-25 software. The results' statistical significance level ($p<0.001$ and $p<0.05$) was observed.

RESULTS

Nutrition plays a significant role in preventing and managing any disorder in the body. The mechanisms of various nutrients are specifically linked with the different

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conditions. Banana peel extract was also used to improve health as it can manage numerous dangerous diseases, i.e., polycystic ovary syndrome, hyperlipidemia, hyperglycemia, and other skin-linked diseases. In the current study, banana peel extracts anti-hyperglycemic and antioxidant attributes are prepared with acetone, methanol, and ethanol. Then the anti-oxidative properties of banana peel extract against diabetes were assessed by using rat modeling. The mean results for the impact of solvents, i.e., acetone methanol and ethanol, on the antioxidant activity of different banana peel extracts are shown in Table 4. The antioxidant properties were assessed by measuring DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, total phenolic contents (TPC), and total flavonoid contents (TFC).The TPC contents of banana peel extract were observed highest in methanol (54.78 ± 0.69 mg GAE/g), followed by ethanol (30.44 ± 0.64 mg GAE/g) and acetone (20.14 ± 0.32 mg GAE/g) solutions. The highest DPPH and TFC contents of banana peel extract were also noticed in methanol solution (74.20 ± 0.98 & 39.48 ± 0.37%), followed by ethanol and acetone. The highest values of banana peel extract in methanol solution were due to the reduced pressure process causing the increment in TPC, DPPH, and TFC contents, as shown in Table 1.

Solvent	TPC	DPPH	TFC	Mean ± SD
Ethanol	30.44 ± 0.64	52.87 ± 1.03	34.56 ± 0.87	39.29 ± 0.84
Methanol	54.78 ± 0.69	74.20 ± 0.98	39.48 ± 0.37	56.15 ± 0.68
Acetone	20.14 ± 0.32	30.53 ± 0.70	21.40 ± 0.17	24.02 ± 0.39

Table 1: Antioxidants concentrations in banana peel extract. Values are expressed as means ± standard deviation

The glucose present in the blood is known as blood glucose produced from the eaten food. It provides the energy to the whole body's cells for their proper functions. The excess and deficiency of blood glucose are linked with various critical diseases, i.e., higher blood glucose concentrations can lead to diabetes mellitus. The mean values of glucose levels were observed to be higher on the 2nd day, followed by the 7th day, 14th day, and 21st day. However, the most negligible value was observed at 0 day. The results depicted that banana peel extracts significantly affected blood glucose levels at 0, 2nd, 7th, 14th, and 21st day. Multiple blood glucose levels were compared on various days, and the HSD Tucky test was applied to assess the variations and significance levels. Each group was compared to the remaining groups on the 0, 2nd, 7th, 14th and 21stdays. The control group was significantly different from the ripe at 0 day. At the same time, a non-significant difference was observed between the control and other groups. Moreover, there was a significant difference in control and all other groups (except ripe) on the 2nd, 7th, 14th, and 21st day. The positive, ripe, unripe, and over-ripe compression was also analyzed for the other groups, respectively, as shown in Table 2.

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(I) Study Groups	(J) Study Groups	Day 0		Day 2		Day 7		Day 14		Day 21	
		Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.
Control	Positive	-2.20	0.991	-143.60	0.055	-190.00	0.001	-250.80	0.000	-286.40	0.000
	Unripe	6.80	0.633	-376.00	0.000	-351.00	0.000	-315.80	0.000	-250.40	0.000
	Ripe	24.00	0.001	-176.00	0.014	-116.00	0.069	-88.80	0.041	-11.40	0.981
	Over Ripe	-5.60	0.776	-188.8	0.008	-161.00	0.007	-149.80	0.000	-97.40	0.001
Positive	Control	2.20	0.991	0143.60	0.055	190.00	0.001	250.80	0.000	286.40	0.000
	Unripe	9.00	0.371	-232.40	0.001	-161.00	0.007	-65.00	0.199	36.00	0.443
	Ripe	26.20	0.000	-32.40	0.962	74.00	0.396	162.00	0.000	275.00	0.000
	Over Ripe	-3.40	0.954	-45.20	0.883	29.00	0.952	101.00	0.017	189.00	0.000
Unripe	Control	-6.80	0.633	376.00	0.000	351.00	0.000	315.80	0.000	250.40	0.000
	Positive	-9.00	0.371	232.40	0.001	161.00	0.007	65.00	0.199	-36.00	0.443
	Ripe	17.20	0.015	200.00	0.005	235.00	0.000	227.00	0.000	239.00	0.000
	Over Ripe	-12.40	0.117	187.20	0.008	190.00	0.001	166.00	0.000	153.00	0.000
Ripe	Control	-24.00	0.001	176.00	0.014	116.00	0.069	88.80	0.041	11.40	0.981
	Positive	-26.20	0.000	32.40	0.962	-74.00	0.396	-162.00	0.000	-275.00	0.000
	Unripe	-17.20	0.015	-200.00	0.005	-235.00	0.000	-227.00	0.000	-239.00	0.000
	Over Ripe	-29.60	0.000	-12.80	0.999	-45.00	0.805	-61.00	0.250	-86.00	0.004
Over Ripe	Control	5.60	0.776	188.80	0.008	161.00	0.007	149.80	0.000	97.40	0.001
	Positive	3.40	0.954	45.20	0.883	-29.00	0.952	-101.00	0.017	-189.00	0.000
	Unripe	12.40	0.117	-187.2000*	0.008	-190.00	0.001	-166.00	0.000	-153.00	0.000
	Ripe	29.60	0.000	12.80000	0.999	45.00	0.805	61.00	0.250	86.00	0.004

Table 2: Multiple comparisons of blood glucose on days 0, 2nd, 7th, 14th, and 21st between study groups by applying the Tucky HSD test

The renal functions were tested on the 21st day of the study by assessing serum creatinine, serum urea, and blood urea nitrogen (BUN). A non-significant difference in serum creatinine was observed between the control and ripe (p-value = 0.419) groups, while significant variations were observed between and within the rest of the groups. The non-significant differences in serum urea were observed between the control and Ripe (p-value = 0.589) and positive and unripe (p value= 0.525) groups. At the same time, all other groups showed significant differences between and within the groups, as shown in Table 3. The BUN values were deemed to be non-significant between control and ripe (p-value = 0.715), positive and unripe (p-value = 0.271) and unripe and over-ripe (p-value = 0.186). However, the differences in other groups were observed to be significant.

(I) Study Groups	(J) Study Groups	Serum Creatinine		Serum Urea		BUN	
		Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.
Control	Positive	-3.52	0.000	-24.00	0.000	-15.40	0.000
	Unripe	-2.88	0.000	-21.00	0.000	-11.80	0.000
	Ripe	-0.26	0.419	-2.80	0.589	-2.20	0.715
	Over Ripe	-1.64	0.000	-13.40	0.000	-7.80	0.002
Positive	Control	3.52	0.000	24.00	0.000	15.40	0.000
	Unripe	0.64	0.003	3.00	0.525	3.60	0.271
	Ripe	3.25	0.000	21.20	0.000	13.20	0.000
	Over Ripe	1.88	0.000	10.60	0.000	7.60	0.002
Unripe	Control	2.88	0.000	21.00	0.000	11.80	0.000
	Positive	-0.64	0.003	-3.00	0.525	-3.60	0.271
	Ripe	2.61	0.000	18.20	0.000	9.60	0.000
	Over Ripe	1.24	0.000	7.60	0.006	4.00	0.186
Ripe	Control	0.27	0.419	2.80	0.589	2.20	0.715
	Positive	-3.25	0.000	-21.20	0.000	-13.20	0.000
	Unripe	-2.61	0.000	-18.20	0.000	-9.60	0.000
	Over Ripe	-1.37	0.000	-10.60	0.000	-5.60	0.031
Over Ripe	Control	1.64	0.000	13.40	0.000	7.80	0.002
	Positive	-1.88	0.000	-10.60	0.000	-7.60	0.002
	Unripe	-1.24	0.000	-7.60	0.006	-4.00	0.186
	Ripe	1.37	0.000	10.60	0.000	5.60	0.031

Table 3: Multiple comparisons of serum Creatinine at day 21 between study groups by applying the Tucky HSD test

The liver function tests were conducted on the study's last day (21st). The serum obtained from the rats of all groups was tested for the analyses of total bilirubin, ALT, Alkaline Phosphatase, albumin, and total protein. The results indicated that a statistical significant difference was observed between and within the groups. Moreover, an individual comparison was also conducted between the groups, as shown in Table 4. The non-significant differences in total bilirubin were observed as non-significant among the control and ripe group (P-value = 0.989) positive and unripe group (p-value = 0.752). In contrast, all other groups showed significant variations among them. The ALT variations were noticed non-significant between control and ripe (p-value = 1.000), positive and unripe (p-value = 0.305), unripe and over-ripe (0.769). At the same time, significant variations were

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observed in all remaining groups. The differences in alkaline phosphatase concentrations were non-significant between control and ripe group (p-value = 0.297), positive and unripe (p-value = 0.122), and over ripe and unripe (p-value = 0.104). Moreover, the non-significant differences in albumin concentrations were observed in unripe and over-ripe (p-value = 0.252), and ripe and over-ripe were observed (p = 0.670). Total protein concentrations in control and ripe (p-value = 0.574), and positive and unripe (p-value = 0.992) was non-significant. However, the remaining groups showed significant variations in serum concentrations of alkaline phosphatase, albumin, and total proteins.

(I) Study Groups	(J) Study Groups	Day 0		Day 2		Day 7		Day 14		Day 21	
		Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.	Mean Difference (I-J)	Sig.
Control	Positive	-1.41	0.000	-25.60	0.000	-86.60	0.000	2.64	0.000	3.00	0.000
	Unripe	-1.26	0.000	-19.80	0.000	-75.00	0.000	1.78	0.000	2.85	0.000
	Ripe	-0.06	0.989	0.60	1.000	-9.20	0.297	0.80	0.070	-0.52	0.574
	Over Ripe	-0.82	0.000	-16.40	0.000	-63.00	0.000	1.18	0.004	0.92	0.098
Positive	Control	1.41	0.000	25.60	0.000	86.60	0.000	-2.64	0.000	-3.00	0.000
	Unripe	0.15	0.752	5.80	0.305	11.60	0.122	-0.85	0.047	-0.15	0.992
	Ripe	1.35	0.000	26.20	0.000	77.40	0.000	-1.84	0.000	-3.52	0.000
	Over Ripe	0.59	0.001	9.20	0.035	23.60	0.000	-1.458	0.000	-2.08	0.000
Unripe	Control	1.26	0.000	19.80	0.000	75.00	0.000	-1.78	0.000	-2.85	0.000
	Positive	-0.15	0.752	-5.80	0.305	-11.60	0.122	0.86	0.047	0.15	0.992
	Ripe	1.20	0.000	20.40	0.000	65.80	0.000	-0.98	0.019	-3.37	0.000
	Over Ripe	0.44	0.018	3.40000	0.769	12.00	0.104	-0.60	0.252	-1.93	0.000
Ripe	Control	0.06	0.989	-0.60	1.000	9.20	0.297	-0.80	0.070	0.52	0.574
	Positive	-1.35	0.000	-26.20	0.000	-77.40	0.000	1.84	0.000	3.52	0.000
	Unripe	-1.20	0.000	-20.40	0.000	-65.80	0.000	0.98	0.019	3.37	0.000
	Over Ripe	-0.76	0.000	-17.00	0.000	-53.80	0.000	0.38	0.670	1.44	0.004
Over Ripe	Control	0.82	0.000	16.40	0.000	63.00	0.000	-1.18	0.004	-.92	0.098
	Positive	-0.59	0.001	-9.20	0.035	-23.60	0.000	1.46	0.000	2.08	0.000
	Unripe	-0.44	0.018	-3.40	0.769	-12.00	0.104	0.60	0.252	1.93	0.000
	Ripe	0.76	0.000	17.00	0.000	53.80	0.000	-0.38	0.670	-1.44	0.004

Table 4: Multiple comparisons of total bilirubin, ALT, Alkaline phosphate, Albumin, and total Proteins at day 21 between study groups by applying Tucky HSD test

DISUCSSION

The banana peel extracts were utilized to evaluate their antioxidant attributes and capability to attenuate diabetes. The antioxidant properties were assessed by measuring the DPPH, total phenolic contents (TPC), and total flavonoid contents (TFC) in ethanol, methanol, and acetone solutions. The results exhibited that TPC, DPPH and TFC values were highest in methanol solutions. The results of Kabir et al., were in line with our study. He reported that banana peel showed that the TPC, TFC, and DPPH values were 53.80 ± 2.88 mg GAE/g DM, 16.44 ± 1.45mg QE/g DM, and 79.07±3.70% in hexane solution [13]. The results of DPPH were also in consistency González-Montelongo et al., [14] and Rebello et al., [15]. The maximum effect of banana leaf extract was observed in ripe and over-ripe groups compared to other groups. They reduced the blood glucose levels in serum, suggesting the reducing and preventive effect of banana peel extracts in diabetic rats. The results were in line with the study of Navghare and Dhawale [16]. They demonstrated that rat groups treated with extract of Musa cavendish and Musa acuminate significantly reduced their blood glucose levels ($p < 0.01$) after 15 minutes than the control group. Moreover, in a hypoglycemic study, the extracts of Musa paradisiacal peels (EMP 500 mg/kg) significantly reduced glucose levels at 120 minutes. The glucose levels decreasing trends were in line with the study of Indriawati and Atiyah, who reported that banana peel extract with concentrations of 400 mg/kg BW, 200 mg/kg BW, and 100 mg/kg BW reduced the blood glucose levels from 337.75 ± 44.9 to 203.01 ± 47.6 mg/dl, 245.83 ± 6.9 to 144.32 ± 42.9 mg/dl and 275.98 ± 50.1 to 171.75 ± 42.4 mg/dl,

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respectively. The lower concentrations of creatinine in serum indicated that the banana peel extract improves the functionality of the kidneys [17, 18]. The banana peel extracts improved renal functionality as they helped in management of serum creatinine, serum urea, and BUN levels in the normal range during diabetes in the rats. Ahmed et al., showed a significant reduction in serum creatinine by consuming the inner banana peels in the nicotinamide/streptozotocin-induced diabetic rats [19]. The results of another study by Vijay et al., were in line with the current study, which resulted from that diabetes-induced animals given the extract of Musa acuminata peel with concentrations of 200 and 400 mg/kg reduced the serum creatinine levels ($p<0.01$) [20]. Lousek et al., reported that the biomass of green banana enhanced the serum urea (37.76 ± 4.13 mg/dL), which was greater than the reference value [21]. Rigueira et al., resulted that banana peel maintains the blood urea nitrogen to the value of 16.22 mg/dL [22]. These results were at par with the results of the current study. The unripe, ripe and over ripe banana peels effected serum concentrations of alkaline phosphatase, albumin, and total proteins in rats significantly leading the reduction in diabetes levels. The study of Vijay et al., in the coherence of our results, reported that the extract of Musa acuminate peel (EMA) with concentrations of 200 and 400 mg/kg caused a significant increase in serum albumin levels in diabetes-induced rats [20]. Though, extract Musa acuminata peel (400 mg/kg) caused a significant increase in total protein levels in serum.

CONCLUSIONS

The unripe, ripe, and over-ripe banana peel played a prominent role in the reduction of glucose levels among diabetic rats. It also helped maintain serum creatinine, blood urea, and blood urea nitrogen levels, improving renal functionality. Moreover, it improved the liver's functional attributes. The current study suggested that the utilization of banana peels has dual benefits as it will help in environmental waste management and can play a beneficial role in treating diabetes and various other ailments.

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