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Biochemical Changes of Ethanolic Leaf Extract *Psidium guajava* of on Streptozotocin Induced Diabetic Rats

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ABSTRACT

Background: Diabetes mellitus (or diabetes) is a chronic, lifelong condition that affects the body's ability to use the energy found in food due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance. The work is aimed at evaluating the biochemical changes on ethanolic leaf extract *Psidium guajava* of in streptozotocin induced diabetic albino rats.

Methods: A total of seventy (70) ratus norvegicus rats were randomly divided into 5 groups of seven rats in each group. Group A served as normal control and received normal saline (2 ml/kg body weight). Group B was treated Streptozotocin, Group C was treated with Streptozotocin and 200 mg/kg body weight of extract, D was treated with Streptozotocin and 400 mg/kg body weight of extract and E was treated Streptozotocin and 5 mg/kg body weight of metformin. Glucose level, levels of renal function status biomarkers and hepatic function biomarkers were determined at the end of the study (28 days).

Result: Acute toxicity test revealed no mortality at maximum dose of 5000 mg/kg which suggests that test substance is safe for the doses used for this study. The result in liver enzymes shows significant ($p < 0.05$) increases in AST, ALP and ALP when compared to the normal control but showed reduction in the protein level. Group B showed significant ($p < 0.05$) increase in urea when compared with the normal control while Group C (200 mg/kg), Group D (400 mg/kg) and Group E (5 mg/kg metformin) showed decreases in the levels of Creatinine when compared to normal control rats. All doses of the plant extract significantly ($p < 0.05$) mitigated change; the reversals at 200 mg/kg body weight of the extract compared well ($p < 0.05$) with their respective non- streptozotocin normal saline treatment control animals in 400 mg/kg of the parameters investigated.

Conclusion: Conclusively, the study demonstrated that ethanoic leaf extract of *P. guajava* can reverse the hyperglycaemia associated with diabetes mellitus, when compared with a known standard drug metformin. There is need for further research's which will be aimed at isolating and packaging the bioactive compounds responsible for this effect into finished pharmaceutical product.

KEYWORDS

Psidium guajava, Diabetes mellitus, Streptozotocin, Acute toxicity, Renal function

INTRODUCTION

Diabetes mellitus is the commonest endocrine disorder that affects more than 100 million people worldwide (6% of the population) and in the next 10 years it may affect about five times more people than it does now (Shakeera *et al.*, 2013). Diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Rajesh *et al.*, 2010). It is a consequence of hereditary and environmental factors which its symptoms include polyuria, polydipsia and polyphagia (Pasupuletin *et al.*, 2013). The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable. Nigeria, like most developing countries is richly blessed with different herbs, shrubs, and trees. These indigenous plants have been the earliest

companion of mankind, providing food, shelter and serving humanity in curing different diseases and healing of injuries (Jaleel *et al.*, 2008). The quest for a reliable and affordable means of treating and management of diabetes mellitus has led to the venture into plant kingdom in search of antidiabetic medicinal plants. Plants used in traditional medicine to treat diabetes mellitus represent a valuable alternative for the control of this disease. Alternative strategies to the current modern conventional therapy of diabetes mellitus are urgently needed, because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the high cost and poor availability of the modern therapies for many rural populations in developing countries.

Some medicinal uses of *Psidium guajava* includes anti-viral, anti-diarrhal, anti-bacterial and antioxidant properties. Shakeera *et al.*, 2013 posited the antihyperglycemic and antihyperlipidemic potentials of *P. guajava* in streptozotocin-induced diabetic rats. Chronic hyperglycaemia during diabetes causes glycation of body proteins that in turn leads to secondary complications affecting eyes, kidneys, nerves and arteries. Kidney is the second organ most frequently affected by any compound after the liver. Thus, the present study was carried out to evaluate effect of *P. guajava* ethanolic leaf extract on the renal function status biomarkers and some biochemical in streptozotocin induced diabetic rat model.

MATERIALS AND METHODS

Preparation of the Extract

Fresh leaves of *P. guajava* were collected in Abia State University Uturu, beside the university cafeteria. Plant material was dried under shade at room temperature, pulverized by a mechanical grinder and sieved through 40 meshes. The powdered material (100 g) was extracted with 3 volumes of 95% ethanol by hot continuous percolation method in a Soxhlet apparatus. The extract was then concentrated and dried under reduced pressure. The ethanol free semi solid mass obtained (13.65 g) was used for the experiment.

Experimental Animals

Male albino rats of 6-8 weeks age, weighing 100-250 g, were used for this study. The animals were purchased from the animal house of University of Nigeria, Nsukka (UNN), Enugu State. The animals were kept in clean, dry and well-ventilated cages, with 12 h: 12 h light-dark cycle at $25 \pm 2^\circ$ temperature and 45-55% relative humidity. They were fed with standard pellet diet and water was given *ad libitum*. The animals were acclimatized for two weeks before the commencement of the investigation. This study was carried out in the animal house of department of biochemistry of Abia State University Uturu and this study was approved by the Institutional Ethical Committee.

Induction of Diabetes in Rats

The rats were injected intraperitoneal with streptozotocin monohydrate (Span Chemical Co., Mumbai) dissolved in sterile normal saline at a dose of 120 mg kg⁻¹ b.wt. The rats were kept for 15 days to stabilize the diabetic condition. Only rats with a fasting blood glucose level of at least 200 mg/d (Ji *et al.*, 2006). The LD₅₀ Acute Toxicity Test was carried out by Lorke method (1983), Analysis of the liver function enzymes was done by the method described by Reitman and Frankel, (1957).

Blood Collection

The blood samples were collected directly from ventricle of heart.

The blood samples which were collected in heparinised tubes were then centrifuged at 3000 g for 15 minutes. The clear serum obtained was used for the analysis of glucose and insulin.

Experimental Design

A total of seventy (70) ratus norvegicus rats were randomly divided into 5 groups of seven rats in each group. Group A served as normal control and received normal saline (2 ml/kg body weight). Group B was treated Streptozotocin, Group C was treated with Streptozotocin and 200 mg/kg body weight of extract, D was treated with Streptozotocin and 400 mg/kg body weight of extract and E was treated Streptozotocin and 5 mg/kg body weight of metformin. Glucose level, levels of renal function status biomarkers and hepatic function biomarkers were determined at the end of the study (28 days).

Statistical Analysis

Data represent the mean \pm Standard Deviation (S.D.) of the indicated number of experiments. Statistical analysis was performed using Onaway Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT) by using statistical Package of Social Science (SPSS) version 12.0 for windows. P values <0.05 were considered as level of significance.

RESULTS

Table 1 shows the mean weight of experimental animals from Day one to fourteenth day of acclimatization. It shows progressive increase in weight of the albino rats which indicates that they are healthy, well fed and had no appetite impediment.

Table 2 shows result of acute toxicity test of *P. guajava* leaf extract: no mortality was observed in the two phases of the test which shows that the test substance is safe for the concentrations used for this study.

Table 3 shows effect of *P. guajava* ethanolic leaf extract on the body weight (g) in streptozotocin-induced diabetic rats. The weight of Group A increased progressively throughout the study. Group B showed reduction in body weight. Group C revealed progressive increase in body weight till the fourth week of the study. Group D and Group E showed increase in body weight as well.

Table 4 shows effect of *P. guajava* ethanolic leaf extract on glucose level (mg/dl) in streptozotocin-induced diabetic rats. Group A shows glucose level within same range showing no significant increases while the glucose level of Group B (Diabetic rats) rose significantly. Group C, D and E followed same trend in which there was a sudden increased followed by significant reductions in the glucose level.

Table 5 shows the effect of *P. guajava* ethanolic leaf extract on renal function status biomarkers in streptozotocin-induced diabetic rats. Group B showed increase when compared with the normal control while Group C (200 mg/kg of Extract), Group D (400 mg/kg of Extract) and Group E (5 mg/kg metformin) showed decreases to the level of the normal control rats.

Table 6 shows the result of effect of *P. guajava* ethanolic leaf extract on serum liver function status biomarkers of streptozotocin-induced diabetic rats. Group B showed significant increases in AST, ALP and ALP when compared to the normal control but showed reduction in the protein level. Group B showed but Group C and Group D as well as Group E shows significant reduction in AST, ALP and ALP and then increases in protein.

Table 1: Mean weight of Albino rats from Day one to fourteenth day of acclimatization

Groups	1 st Day (g)	Fourth day (g)	Tent day (g)	Fourteenth day (g)
Group A	95.75	96.98	168.92	170.63
Group B	159.79	154.71	179.57	184.20
Group C	71.46	69.49	80.90	90.30
Group D	134.8	130.41	151.40	160.20
Group E	148.48	143.48	168.90	170.10

Table 2: Result of Acute Toxicity study (LD₅₀)

Groups	Dosage	Mortality	Behaviour
Phase 1			
Group 1	10	0/3	Scratching of mouth, moving around the cage, shaking of head, quietness for about 5 seconds, mouth scratching continued after 2 minutes and then calmness
Group 2	100	0/3	Shaking of head, running around the cage, scratching 12-month, calmness after 30 seconds.
Group 3	1000	0/3	Quietness for about 2 seconds, slow movement after 5 seconds, extremely calmness after 1 minute, scratching of mouth and calmness after 2 minutes.
Phase 2			
Group 1	1600	0/1	Running around the cage, scratching of mouth, shaking of head, apnea, calmness after 3 minutes very slow in movement, scratching of mouth after 15 seconds, shaking of head and then calmness after 4 minutes.
Group 2	5000	0/1	Very active in movement, shaking of head after 2 minutes calmness for 30 seconds, scratching of mouth, apnea and then calmness.

Table 3: Effect of *Psidium guajava* ethanolic leaf extract on the body weight (g) Streptozotocin induced diabetic rats

	Week 1	Week 2	Week3	Week 4
Treatment	Weight (g)	Weight (g)	Weight (g)	Weight (g)
Group A (Normal control)	165.00 ^d ± 20.60	167.00 ± 18.60	171.00 ± 20.70	180.00 ± 15.30
Group B (Diabetic control)	188.30 ^a ± 25.00	183.30 ^b ± 15.30	176.70 ± 32.20	162.10 ± 14.20
Group C (200 mg/kg of Extract)	93.30 ± 23.10	100.30 ± 17.90	120.00 ± 14.10	128.30 ^a ± 13.20
Group D (400 mg/kg of Extract)	180.00 ^c ± 28.30	185.00 ^a ± 7.10	194.00 ± 14.10	200.40 ^b ± 20.00
Group E (Metformin)	175.00 ^c ± 14.10	177.50 ^c ± 17.70	189.00 ± 14.10	199.10 ± 10

Results are mean + SD of data at (p<0.05). Mean with different superscripts in the same column are significantly different (p<0.05))

Table 4: Effect of *Psidium guajava* ethanolic leaf extract on glucose level (mg/dl) in induced diabetic rats

	Week 1	Week 2	Week 3	Week 4
Treatment	Glucose level (mg/dl)	Glucose level (mg/dl)	Glucose level (mg/dl)	Glucose level (mg/dl)
Group A (Normal saline)	83.40 ± 7.60	90.00 ± 8.30	98.00 ± 14.20	100.00 ± 13.20
Group B (Diabetic control)	88.70 ± 7.0	236.30 ^a ± 199.0	372.7 ^a ± 157.3	400.10 ^a ± 147.00
Group C (200 mg/kg of Extract)	80.30 ± 7.00	143.50 ^b ± 29.00	120.00 ^c ± 18.70	105.10 ^b ± 17.00
Group D (400 mg/kg of Extract)	82.00 ± 71	143.7 ^d ± 40.20	110.50 ^d ± 9.20	102.40 ^c ± 9.00
Group E (metformin)	81.00 ± 5.70	151.0 ^c ± 59.40	124.50 ^b ± 26.20	98.60 ± 30.10

Results are mean + SD of data at (p<0.05. mean with different superscripts in the same column are significantly different (p<0.05))

Table 5: Effect of *Psidium guajava* ethanolic leaf extract on Renal function status biomarkers in streptozotocin-induced diabetic rats

Treatment	Urea (mg/dL)	Creatinine (mg/dL)
Group A (normal saline)	12.05 ± 1.04	0.73 ± 0.12
Group B (Diabetic control)	29.63 ^a ± 0.84	2.45 ^a ± 0.35
Group C (200 mg/kg of Extract)	13.06 ^b ± 1.14	0.84 ± 0.36
Group D (400 mg/kg of Extract)	12.84 ± 1.84	0.75 ± 6.24
Group E (5 mg/kg metformin)	13.05 ^c ± 1.56	0.74 ± 0.11

Results are mean + SD of data at (p<0.05. mean with different superscripts in the same column are significantly different (p<0.05)).

Table 6: The Effect of *Psidium guajava* ethanolic leaf extract on serum liver function status biomarkers of streptozotocin-induced diabetic rats

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Protein(mg/dL)
Group A (Normal saline)	88.40 ± 12.44	46.60 ± 14.60	56.97 ± 0.18	7.93 ± 0.16
Group B (Diabetic control)	129.63 ^a ± 1 0.84	90.20 ^a ± 0.35	102.02 ^a ± 0.19	5.02 ± 0.19
Group C (200 mg/kg of Extract)	73.06 ± 1.14	60.94 ^b ± 0.36	80.84 ^b ± 0.13	6.84 ± 0.13
Group D (400 mg/kg of Extract)	71.84 ± 1.84	55.38 ^c ± 6.24	65.54 ^c ± 0.24	7.54 ± 0.24
Group E (Metformin)	85.05 ± 1.56	51.05 ^d ± 0.11	62.96 ^c ± 0.38	7.96 ± 0.38

Results are mean + SD of data at (p<0.05. mean with different superscripts in the same column are significantly different (p<0.05); Alkaline Phosphatase (ALP); Alanine Transaminase (ALT); Aspartate Transaminase (AST))

DISCUSSION

Plant-derived products lack significant side effects when administered properly. Therefore, they are harnessed for the efficacies of bioactive compounds present in them that aids in treatment and management of ailments including diabetes mellitus (Grovers *et al.*, 2000). Diabetes is a common endocrine disorder and a clinical syndrome characterized by inappropriate hyperglycaemia caused by a relative or absolutely deficiency of insulin or by a resistance to the action of insulin at the cellular level (Wadkar *et al.*, 2008). The present study was to ascertain the effect of ethanolic *P. guajava* leaf extract on streptozotocin-induced diabetic rats, as it has been reported to possess flavonoid and terpenoids which may help to hasten the natural healing process and consequently improve glycemic control in diabetes. Table 1 shows the mean weight of experimental animals from Day one to fourteenth day of acclimatization. It shows progressive increase in weight of the albino rats which indicates that they are healthy, well fed and had no appetite impediment. Acute toxicity test of ethanolic *P. guajava* leaf extract revealed no mortality at maximum dose of 5000 mg/kg which suggests that test substance is safe for the doses used for this study.

Table 3: shows effect of *P. guajava* ethanolic leaf extract on the body weight (g) in streptozotocin-induced diabetic rats. The weight of Group A increased progressively throughout the study. Group B showed reduction in body weight. Group C revealed progressive increase in body weight till the fourth week of the study. Group D and Group E showed increase in body weight. There was a reduction of body weight in streptozotocin diabetic rats but this was reversed as increase in body weight of *P. guajava* leaf extract was observed, this increase was followed the same pattern as seen in metformin treated diabetic rats. This may be attributed to the ability of the extract to ameliorate diabetes.

Table 4 shows effect of *P. guajava* ethanolic leaf extract on glucose level (mg/dl) in Streptozotocin induced diabetic rats. Group A shows glucose level within same range showing no significant increases while the glucose level of Group B (Diabetic rats) rose significantly. Group C, D and E followed same trend in which there was an initial sudden increase followed by significant reductions in the glucose level. Insulin deficiency occurs in streptozotocin induced-diabetic rats leading to alterations in the carbohydrate metabolism such as elevated blood glucose and reduced level of insulin release, by destroying the β -cells of the islets of Langerhans (Rosenberg, 1995). In our study we noticed that *P. guajava* ethanolic leaf extract reversed these effects bringing the glucose level to normal and its effect was also comparable to the observation found in metformin (A hypoglycaemic drug). This is an indication that the aim of treating diabetic patients which is to reduce the glucose level was achieved (Une Hemant *et al.*, 2014). The ability of the plant to reduce the glucose level may be attributed to its phytochemical constituents such as phenols, alkaloids, saponins, glycosides and carbohydrate as reported by Lincy *et al.*, 2016.

Table 5 shows the effect of *P. guajava* ethanolic leaf extract on renal function status biomarkers in Streptozotocin induced diabetic rats. Group B showed significant ($p < 0.05$) increase in urea when compared with the normal control while Group C (200 mg/kg of Extract), Group D (400 mg/kg of Extract) and Group E (5 mg/kg metformin) showed decreases in the levels of Creatinine when compared to normal control rats. Kidney is an important organ that maintains optimum composition of body fluid by acidification of urine and removal of metabolic wastes such as creatinine and urea. When the integrity of the kidney is compromised as in kidney diseases, high concentration of creatinine and urea is found in the blood (Wadker *et al.*, 2008). Contrarily, there was reduction in these, parameter in the *P. guajava* treated group which can also be compared to the reduction observed in metformin treated group. This suggests that there was no significant impairment of the kidney excretory function.

Table 6 shows the result of effect of *P. guajava* ethanolic leaf extract on serum liver function status biomarkers of alloxan-induced diabetic rats. Group B (Diabetic rats) showed significant increases in

AST, ALP and ALP when compared to the normal control but showed reduction in the protein level. Group B showed but Group C and Group D as well as Group E shows significant reduction in AST, ALP and ALP and then increases in protein. The increases of AST, ALP and ALP found in the diabetic rats are as a result of the fact that transaminases are active in the absence of insulin because of increased activity of amino acid in diabetes which accounts for increased gluconeogenesis and ketogenesis in diabetes (Wadker *et al.*, 2008). In our study efficacies of *P. guajava* ethanolic leaf extract and metformin in reduction of the levels of AST, ALP and ALP were recorded. Increase in the Protein concentration decreased in streptozotocin-diabetic rats but on treating with the plant extract and metformin in group C, D, and E respectively an increase in protein was registered. This is an indication that the plant extract reversed the synthetic function of the hepatocytes cells of the liver which were destroyed and their function in turn impaired in the diabetic albino rats.

CONCLUSION

Conclusively, the study demonstrated that ethanoic leaf extract of *P. guajava* can reverse the hyperglycaemia associated with diabetes mellitus, when compared with a known standard drug metformin. There is need for further research's which will be aimed at isolating and packaging the bioactive compounds responsible for this effect into finished pharmaceutical product.

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