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Original Research

Musa sapientum with exercises attenuates hyperglycemia and pancreatic islet cells degeneration in alloxan-diabetic rats

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ABSTRACT

Aim: We tested the hypothesis that administrations of methanolic extracts of *Musa sapientum* sucker (MEMS) with exercises attenuated hyperglycemia in alloxan-diabetic rats. **Materials and Methods:** A total of 40 adult male rats were divided into equal eight groups. Normoglycemic Group A was Control. Alloxan (180 mg/kg, i.p.) was administered to rats in Groups B - H to induce diabetes. Group B (diabetic control) received physiological saline. Groups C - H received MEMS (5 mg/kg), MEMS (10 mg/kg), Glibenclamide (5 mg/kg), MEMS (5 mg/kg) + exercises, MEMS (10 mg/kg) + exercises and Exercises only, respectively. Changes in body weight, blood glucose levels (BGL) and pancreatic histology were evaluated during or at the end of experiment. Body weights and BGL of rats were expressed as mean ± standard deviation and analyzed using the statistical software program SPSS 15. Statistical comparisons were done using the Student's *t*-test for unpaired samples. Differences between groups were determined as significant at $P \leq 0.05$. **Results:** Significantly ($P < 0.05$) decreased bodyweight was observed in B and H compared to A and C - G. Treatment with MEMS significantly ($P < 0.05$) decreased elevated BGL in C and D. Hypoglycemic effect of MEMS appeared enhanced with exercises in F and G. Exercises regimen alone (H) resulted in percentage reduction in BGL lower than those of C - G. Histopathological examinations revealed normal pancreas (A), atrophied islet cells (B), hyperplasia with adequate population of islet cells (C - G), and reduced hyperplasia of islet cells (H). **Conclusion:** MEMS with exercises attenuated hyperglycemia in alloxan-diabetic rats.

KEY WORDS: Alloxan, hyperglycemia, *Musa sapientum,* pancreatic islet cells, rats

INTRODUCTION

Diabetes mellitus characterized by hyperglycemia results from complete or relative deficiency of insulin. It could result from autoimmune insufficient insulin production by pancreatic islet cells or resistance of cells to insulin actions [1]. Insulin insufficiency disrupts water and electrolyte homeostasis due to adverse effects on carbohydrate, protein, and fat metabolism resulting in deranged structural and functional body systems. Diabetes is a global health burden with the estimated 171 million people affected in year 2000 expected to be doubled by 2030 [1]. Current antidiabetic therapeutic regimens (orthodox or complementary alternative medicine) are at best palliative. There is yet no curative treatment for diabetes mellitus, hence it is most relevant that further scientific efforts are made to discover plants with potential hypoglycemic properties in order to identify and produce better therapeutic anti-diabetic drugs.

M. sapientum belongs to the family Musaceae and is a food crop well-grown in villages and towns in Nigeria, and its various parts have been described to possess different medicinal properties [2]. Significant antioxidant properties have been observed in investigations of peel extracts [3], inflorescence and stalk [4] of *M. sapientum*. Its banana pulps [5] and unripe plantain bananas [6] have been reported to have anti-ulcerogenic properties; its seeds possess antioxidant, anti-diarrheal, and anti-microbial activities [7], its flowers possess hypoglycemic activities [8] while its sucker possesses hypoglycemic [9] and anti-ulcerogenic [2] potentials.

During physical activities or exercises, oxygen consumption in working muscles increases several folds (>20-fold) with the muscles making use of stored free fatty acids, triglycerides, and glycogen obtained from the catabolism of adipose tissue triglycerides and liver glucose [10]. Body muscles represent approximately 40% of the mass of the body, hence muscular actions are of great

importance to the control of blood glucose levels (BGL) and prevention of diabetes [11-13]. Intense exercises had been reported to exert positive effects on insulin release and glucoregulation [14]. Furthermore, epidemiological studies showed that the incidence of non-insulin dependent diabetes mellitus negatively correlated with measures of physical activities. This is due to the fact that exercises even at mild levels enhanced the uptake of glucose by cells in apparently normal and insulin-resistant individuals [14]. In addition, it has been reported that some mild cases of diabetes were treatable with exercises and improved diet even in the absence of insulin treatment [14]. Exercises and/or physical activities may, therefore, be of therapeutic relevance to individuals susceptible to diabetes and diabetic patients [10].

The hypoglycemic potentials of *M. sapientum* sucker in alloxan-diabetic rats have been previously noted [9]. However, in further considerations of the possible roles of exercises in the treatment of diabetes and to demonstrate whether or not that exercises could improve the hypoglycemic activities of *M. sapientum* sucker extract; we tested the hypothesis that the administrations of methanolic extracts of *M. sapientum* sucker with exercises attenuated hyperglycemia in alloxan-diabetic male Wistar rats.

MATERIALS AND METHODS

Collection and Authentication of *M. sapientum*

Fresh sucker of *M. sapientum* (10 kg) was collected from a farmland on Olabisi Onabanjo University Campus in Ikenne, Ogun State, Nigeria. The plant's identity was confirmed and authenticated at the Forest Research Institute of Nigeria, Ibadan, Oyo State, assigned the voucher number FHI 108349 and samples were deposited at the herbarium.

Animals

Totally, 40 male adult Wistar rats weighing between 200 g and 250 g and aged 22-25 weeks old were obtained from the colony bred of the Department of Physiology, University of Ibadan, Nigeria. Animals were fed throughout experimental procedures with growers feed produced by Bendel Feed and Flour Mill Limited, Nigeria. The animals were caged and kept under the standard condition in a well-ventilated animal house of the Faculty of Basic Medical Sciences of Olabisi Onabanjo University, Nigeria at the room temperature of 25°C. All rats received water ad libitum and were acclimatized for 2 weeks before the start of experimental procedures. Ethical Approval was obtained from the Ethical Committee of the Faculty of Basic Medical Sciences of Olabisi Onabanjo University, Nigeria, and the experimental procedures were carried out in accordance with the "Principles of laboratory animal care" of NIH publication number 85-23 as revised in 1985.

Preparation of Plant Extracts

Harvested *M. sapientum* suckers were rinsed and chopped into small pieces to increase the surface area for easy and fast drying. The pieces were shade dried at room temperature 25°C-30°C for 2 weeks in order to prevent direct sunlight, which can react with the active ingredients of the plant. This prevented deterioration of the phytochemical constituents of the plant material. Dried pieces of the plant material were powdered. 200 g of the dried sucker was extracted in 70% methanol for 48 h. The extract was then filtered and concentrated using rotary evaporator. The concentrated extract was further dried on a water bath. The total yield of the methanolic extract of *M. sapentium* (MEMS) was 2.61%.

Phytochemical Evaluation

M. sapientum extract was evaluated for the presence of different chemical groups using standard methods as earlier described [2,9].

Experimental Design and Treatment Groups

Totally, 40 rats were weighed, and fasting BGL determined. 5 of these normoglycemic rats were randomly selected and served as the normal control (Group A). Alloxan monohydrate (BDH Chemical Limited, Poole, England) at a dose of 180 mg/kg as determined from previous studies [9,15,16] was injected intraperitoneally to the remaining normoglycemic rats ($n = 35$) to induce diabetes. Rats with fasting BGL ≥ 190.0 mg/dl 72 h after alloxan injection were randomized into seven groups (Groups B - H) of five rats per group and treated as follows:

1. Group B: Physiological saline (diabetic control)
2. Group C: MEMS (5 mg/kg)
3. Group D: MEMS (10 mg/kg)
4. Group E: Glibenclamide (5 mg/kg)
5. Group F: MEMS (5 mg/kg) + exercises
6. Group G: MEMS (10 mg/kg) + exercises
7. Group H: Exercises only

MEMS, glibenclamide, and physiological saline were administered orally for 21 consecutive days. MEMS were administered to rats in Groups E and F, 30 min prior to their engagement in exercises.

Method for Engaging Rats in Physical Activity/Exercises

Rats in Groups E, F, and H were engaged in physical activity or exercises using the modified method of previous studies [Figure 1] [11-13]. Rats were exercised by swimming unaided for 60 min daily for 5 days/week by placing them in a 60 cm deep plastic container at 32 ± 1°C. The rats were trained for the exercise model and were exercised for 60 min/day (5 times a week) for a period of 3 weeks or 21 days (Days 5-26) to ensure adaptation of swimming into the memory of the rats so that the resulting effects on body organs/tissues were no longer due to stress.

Estimation of BGL

Fasting BGL was measured by collecting a drop of blood from the tail of each rat after incision with a sharp blade. The blood was dropped onto dextrostix (Accu-Chek system, Roche, Group, Germany) reagent pad and values read using the microprocessor digital blood glucometer (GlucotrendR2, Accu-Chek system, Roche, Group, Germany). BGL was measured and recorded on days 1, 2, 5, 12, 19, and 26 of experiments.

Histology

Rats were sacrificed under diethyl ether (BDH Chemical Limited, Poole, England) anesthesia at the end of all treatments. The pancreas was removed and immediately fixed in 10% formalin solution. Fine microscopic sections were obtained and mounted on glass slides. The mounted sections were then counterstained with hematoxylin and eosin for light microscopic analyses [9,15,16].

Statistical Analysis

Body weights and BGL of rats were expressed as mean ± standard deviation and analyzed using the statistical software program SPSS 15. Statistical comparisons were done using the Student's *t*-test for unpaired samples. Differences between groups were determined as significant at $P \leq 0.05$.

RESULTS

Phytochemical Analysis

Phytochemical screening of *M. sapientum* sucker revealed the presence of saponins, saponin glycosides, tannins, alkaloids, and indole alkaloids.

Changes in Body Weight

Body weights of rats significantly decreased ($P < 0.05$) in rats belonging to Group B (untreated alloxan-diabetic Group) or alloxan-diabetic rats in Group H treated with exercises only. Body weight increased significantly ($P < 0.05$) in control rats (Group A) and alloxan-diabetic rats in Groups C - G treated with MEMS and exercises or glibenclamide [Figure 2].

Blood Glucose Level

BGL of alloxan-diabetic rats significantly increased ($P < 0.05$) in Group B rats when compared with normoglycemic rats (Group A). Treatment with MEMS, MEMS with exercises, glibenclamide or exercises only significantly ($P < 0.05$) reduced alloxan-induced hyperglycemia as observed in Groups C - H [Figure 3]. Treatment with exercises only (Group H) produced a significantly lower reduction in BGL when compared with glibenclamide-treated rats (Group E). However, a significantly higher reduction in BGL were observed in rats belonging to Groups C, D, F, and G treated with MEMS (with or without exercises) when compared with glibenclamide-treated rats (Group E) [Figure 3]. The best hypoglycemic effect was observed in Group D rats treated with MEMS (10 mg/kg) [Figure 3]. There was no significant difference ($P > 0.05$) in the extent of BGL reduction of diabetic rats treated with MEMS only (Groups C and D) and those treated with MEMS plus exercises (Groups F and G) [Figure 3].

Histological Examination

Histological examination revealed a significant reduction in population of pancreatic islet cells of diabetic control rats of Group B [Figure 4b] when compared with normoglycemic control rats of Group A that showed normal cyto-architectural components of the pancreas with adequate population of pancreatic islet cells [Figure 4a]. Alloxan diabetic rats in

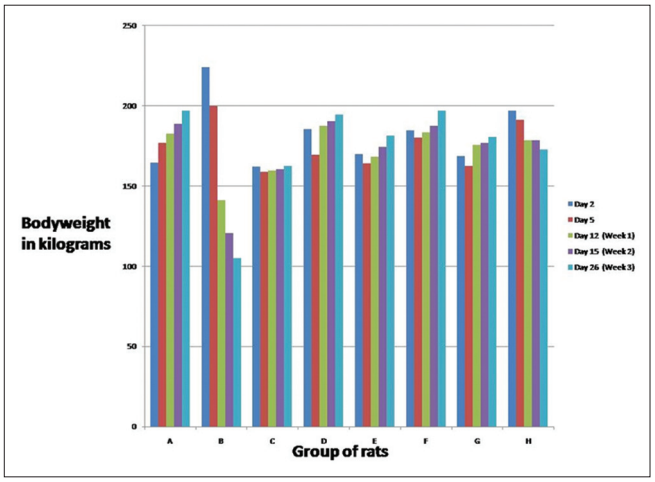

Figure 2: Changes in body weights in kg of rats

Figure 1: Rats of Group F undergoing swimming exercises

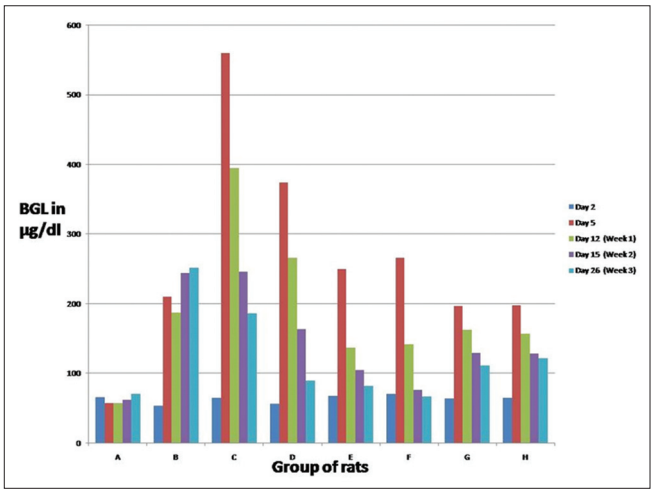

Figure 3: Mean blood glucose levels in µg/dl of Rats

Groups C – G treated with MEMS, MEMS plus exercises or glibenclamide exhibited pancreatic histology similar to those of the normal control [Figures 4a, 5a-c, and 6a-b]. Mild regeneration or hyperplasia of pancreatic islet cells was observed in the rats belonging to Group H [Figure 6c].

DISCUSSION

Pancreatic islet beta cells have the functional responsibility to produce insulin that controls the levels of blood glucose in the body. A dramatic rise in BGL occurs with insufficient release or lack of insulin [1]. The cytotoxic role of alloxan is effected via generation of reactive oxygen species, which lead to the release of highly reactive hydroxyl radicals and increased concentration of cytosolic calcium. The end result is the fast destruction of pancreatic islet beta cells and hyperglycemia [17]. If untreated, the resultant effects could lead to adverse effects on body systems or clinical conditions such as weight loss, neuropathy, impaired renal, and retinal functions [1].

Comparisons of percentage changes in body weights of rats (day 1 vs. day 26) showed statistically significant decreases ($P < 0.05$) in Groups B and H; while statistically significant increases ($P < 0.05$) were observed in control Group A and treated alloxan-diabetic rats of Groups C - G [Figure 2]. This implied that alloxan administrations induced hyperglycemia and weight loss in untreated Alloxan-diabetic rats of Group B. Treatment of hyperglycemia with exercises only could, however, not significantly attenuated hyperglycemia with the resultant weight loss in rats

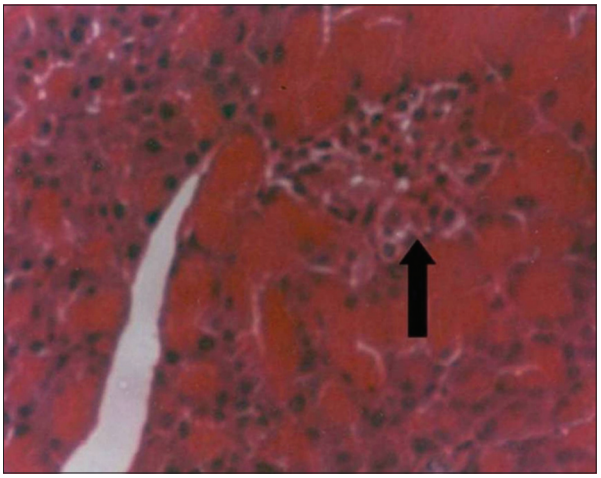

Figure 4a: Photomicrograph sample of pancreas of rats of the normoglycemic Group A that received only physiological saline (hematoxylin and eosin × 200), Solid black arrow indicates normal population of pancreatic islets cells

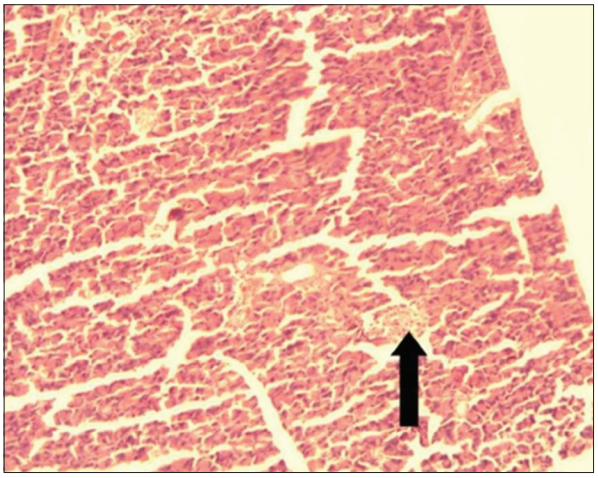

Figure 4b: Photomicrograph sample of pancreas of rats of Group B that received 80 mg/kg/bodyweight of alloxan and treated with only physiological saline (hematoxylin and eosin × 200), Solid black arrow indicates greatly reduced population or atrophy of pancreatic islets cells. This implied loss of beta cells population due to actions of alloxan via production of reactive oxygen species

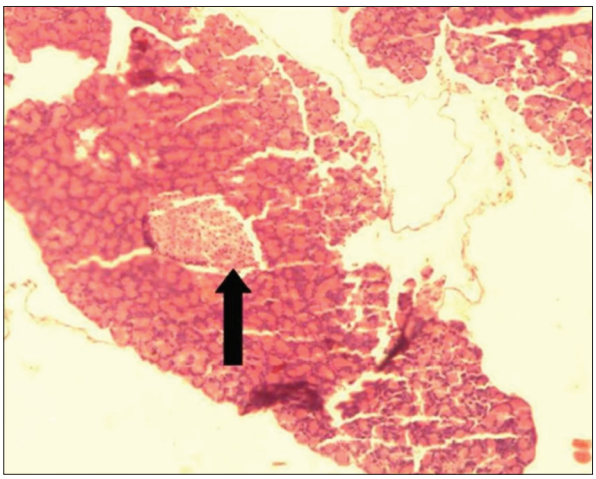

Figure 5a: Photomicrograph sample of pancreas of rats of Group C that received 180 mg/kg/bodyweight of alloxan and treated with 5 mg/kg/bodyweight of methanolic extract of *Musa sapientum* sucker (hematoxylin and eosin × 200), Solid black arrow indicates normal population of pancreatic islets cells. This implied possible regeneration of beta cells population lost due to actions of Alloxan via production of reactive oxygen species

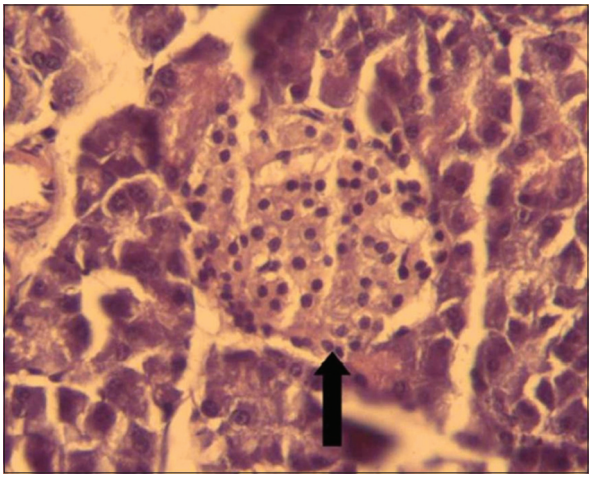

Figure 5b: Photomicrograph sample of pancreas of rats of Group D that received 180 mg/kg/bodyweight of alloxan and treated with 10 mg/kg/bodyweight of methanolic extract of *Musa sapientum* sucker (hematoxylin and eosin × 200), Solid black arrow indicates normal population of pancreatic islets cells. This implied possible regeneration of beta cells population lost due to actions of alloxan via production of reactive oxygen species

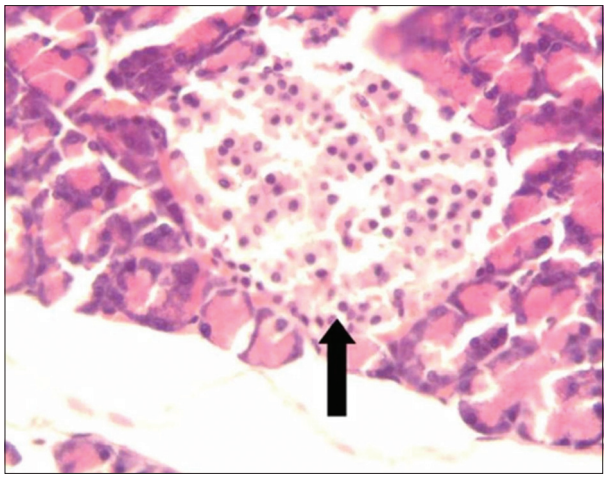

Figure 5c: Photomicrograph sample of pancreas of rats of Group E that received 180 mg/kg/bodyweight of alloxan and treated with 5 mg/kg/bodyweight glibenclamide (hematoxylin and eosin × 200) Solid black arrow indicates normal population of pancreatic islets cells. This implied possible regeneration of beta cells population lost due to actions of alloxan via production of reactive oxygen species

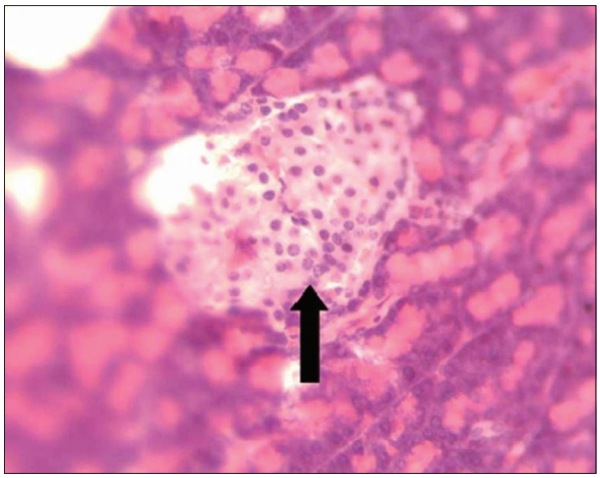

Figure 6b: Photomicrograph sample of pancreas of rats of Group G that received 180 mg/kg/bodyweight of alloxan and treated with 10 mg/kg/bodyweight of methanolic extract of *Musa sapientum* sucker plus 60 min daily exercises for 5 days per week (hematoxylin and eosin × 200), Solid black arrow indicates normal population of pancreatic islets cells. This implied possible regeneration of beta cells population lost due to actions of alloxan via production of reactive oxygen species

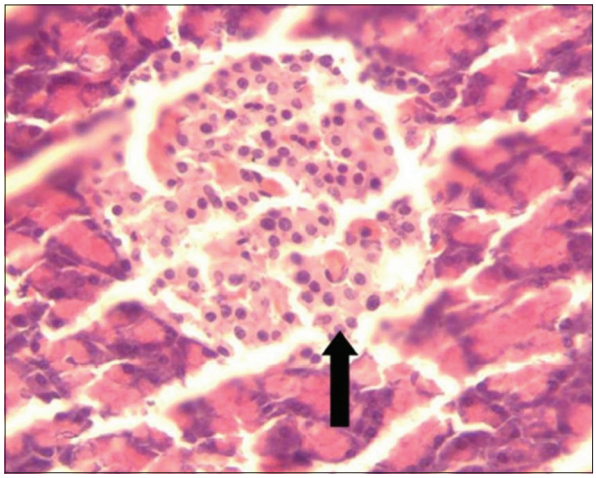

Figure 6a: Photomicrograph sample of pancreas of rats of Group F that received 180 mg/kg/bodyweight of alloxan and treated with 5 mg/kg/bodyweight of methanolic extract of *Musa sapientum* sucker plus 60 min daily exercises for 5 days per week (hematoxylin and eosin × 200), Solid black arrow indicates normal population of pancreatic islets cells. This implied possible regeneration of beta cells population lost due to actions of Alloxan via production of reactive oxygen species

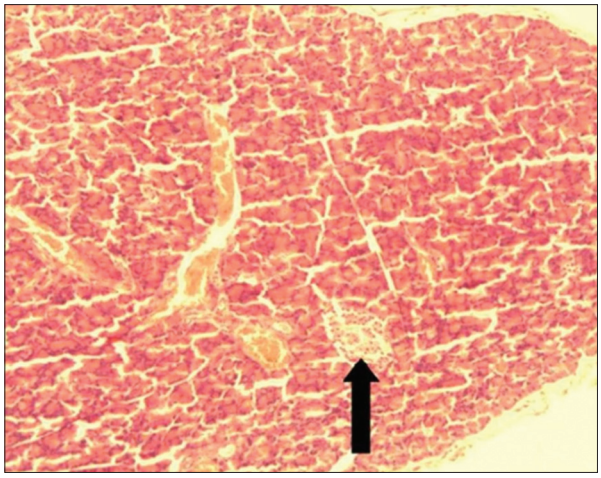

Figure 6c: Photomicrograph sample of pancreas of rats of Group H that received 180 mg/kg/bodyweight of alloxan and treated with swimming exercises only (hematoxylin and eosin × 200), Solid black arrow indicates mild reduction in the population of pancreatic islets cells. This implied mild regeneration of beta cells population lost due to actions of alloxan via production of reactive oxygen species

of Group H. However, the possible resultant weight loss due to hyperglycemia was significantly attenuated in alloxan-diabetic rats treated with 5 and 10 mg/kg/body weight of extract (C and D), 5 and 10 mg/kg/body weight of extract with exercises (F and G), and 5 mg/kg/body weight of glibenclamide (E). Furthermore, the observed effects of the extract on weight loss compared favorably with glibenclamide. Our observations are in agreement with the only previously reported protective effects of *M. sapientum* sucker extracts against weight loss in alloxan-diabetic rats [9].

Histopathological examinations at the end of experimental procedures showed normal pancreas (Group A), atrophied pancreatic islet cells (Group B), hyperplasia with adequate populations of islet cells (Groups C - G), and reduced hyperplasia of islet cells (Group H) [Figures 4a, 5a-c, and 6a-c]. These findings implied that treatments of alloxan-diabetic rats with *M. sapientum* sucker extracts (with or without exercises) were possibly able to aid the regeneration of destroyed pancreatic islet beta cells induced by alloxan in rats. This is in agreement with previously reported pancreatic histoprotective effects of the different parts of *M. sapientum* such as its peel extracts [3], inflorescence, and stalk [4] and sucker extracts [9].

Evaluations of measured BGL on day 1, at the induction of diabetes (day 2) and weekly (weeks 1-3) after the induction of diabetes [Figure 3] showed that alloxan administration resulted

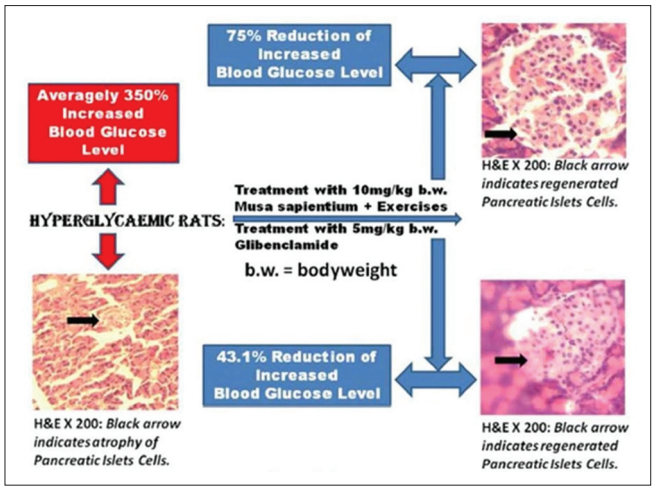

Figure 6d: Graphical abstract shows the hypoglycemic effects of 5 mg/kg bodyweight of glibenclamide (Group E) and 10 mg/kg body weight methanolic extracts of *Musa sapientum* sucker + exercises (Group G) in alloxan diabetic rats

in non-reversible hyperglycemia in untreated alloxan-diabetic rats of Group B. Treatment of hyperglycemia with exercises only significantly attenuated hyperglycemia but at a lower rate when compared with glibenclamide-treated rats. However, hyperglycemia was significantly attenuated in alloxan-diabetic rats treated with extract doses (Groups C and D) and extracts doses with exercises (Groups F and G) comparatively with glibenclamide-treated rats of Group E [Figures 3 and 6d]. The possible hypoglycemic potentials of *M. sapientum* sucker extracts appeared enhanced and improved with exercises. This could suggest that increased muscular activities might have significant reversal roles on hyperglycemia in rats confirming possible supportive anti-diabetic roles of exercises as previously noted [10-13].

Phytochemical screenings of *M. sapientum* sucker showed the ubiquitous existence of tannins, saponins, alkaloids, and glycosides. [2]. The direct mechanism of action of *M. sapientum* sucker is not clear, however, its anti-diabetic properties might be due to its phytochemical components such as tannins, saponins, and alkaloids. Tannins as phenolic compounds promote the status of oxidative stress biomarkers with the ability to scavenge free radicals, which could also have enhanced its anti-diabetic activity [2,18,19]. Saponins possess immunomodulatory, anti-inflammatory, and vasoprotective effects, which could have aided the observed anti-diabetic activity of *M. sapientum* sucker [2,18]. Alkaloids have similarly been noted to possess hypoglycemic properties [19].

CONCLUSIONS

The findings of this study confirmed the hypoglycemic potentials of *M. sapientum* sucker extracts (with or without exercises) in Alloxan-diabetic rats. Furthermore, our findings suggest that treatments with *M. sapientum* sucker extracts promote the restorations of destroyed pancreatic islet cells in alloxan-diabetic rats. This observation could be of relevance in the development of new therapeutic anti-diabetic agents from *M. sapientum* sucker extracts.

REFERENCES

1. Boon AN, Colledge NR, Walker BR, Hunter JA. Davidson's Principles and Practice ofMedicine. 20th ed. India: Churchill Livingstone, Elsevier Limited.; 2006.
2. Akinlolu AA, Ghazali OK, Salau, BA, Jubril O, Omowunmi M. *Musa sapientum* attenuates total antioxidant and lipid profile values in rats with indomethacin-induced gastric ulceration. Cell Membr Free Radic Res 2013;5:236-43.
3. Ramakrishnan B, Selvaraj S, Babu S, Radhakrishnan S, Radhakrishnan N, Palanisamy P. Antioxidant potential of peel extracts of banana varieties (*Musa sapientum*). Food Nutr Sci 2011;2:1128.
4. Jayamurthy P, Aparna B, Gayathri G, Nisha P. Evaluation of antioxidant potential of inflorescence and stalk of plantain (*Musa sapientum*). J Food Biochem 2013;37:2-7.
5. Lewis DA, Fields WN, Shaw GP. A natural flavonoid present in unripe plantain banana pulp (*Musa sapientum* L. var. *paradisiaca*) protects the gastric mucosa from aspirin-induced erosions. J Ethnopharmacol 1999;65:283-8.
6. Prabha P, Karpagam T, Varalakshmi B, Packiavathy AS. Indigenous anti-ulcer activity of *Musa sapientum* on peptic ulcer. Pharmacognosy Res 2011;3:232-8.
7. Hossain MS, Alam MB, Asadujjaman M, Zahan R, Islam MM, Mazumder ME, *et al.* Antidiarrheal, antioxidant and antimicrobial activities of the *Musa sapientum* Seed. Avicenna J Med Biotechnol 2011;3:95-105.
8. Dhanabal SP, Sureshkumar M, Ramanathan M, Suresh B. Hypoglycemic effect of ethanolic extract of *Musa sapientum* on alloxan induced diabetes mellitus in rats and its relation with antioxidant potential. J Herb Pharmacother 2005;5:7-19.
9. Salau BA, Ajani EO, Akinlolu AA, Ekor MN, Soladoye MO. Methanolic extract of *Musa sapientum* sucker moderates fasting blood glucose and body weight of alloxan- induced diabetic rats. Asian J Exp Biol Sci 2010;1:30-5.
10. American Diabetes Association. Physical Activity/Exercises and Diabetes. Diabetes Care 2004. Available from: http://www.care.diabetesjournals.org/content/27/suppl_1/s58.full. doi:10.2337/diacare.27.2007.S58. [Last Accessed on 2015 Apr 30].
11. de Oliveira CA, Luciano E, de Mello MA. The role of exercise on long-term effects of alloxan administered in neonatal rats. Exp Physiol 2005;90:79-86.
12. Rossi DM, Valenti VE, Navega MT. Exercise training attenuates acute hyperalgesia in streptozotocin-induced diabetic female rats. Clinics (Sao Paulo). 2011;66:1615-9.
13. Edite T, Sara N, Frederico T, Flávio R. Regular physical exercise training assists in preventing type 2 diabetes development: Focus on its antioxidant and anti-inflammatory properties. Cardiovasc Diabetol 2011;10:12.
14. Marliss EB, Vranic M. Intense exercise has unique effects on both insulin release and its roles in glucoregulation: Implications for diabetes. Diabetes 2002;51:S271-83.
15. Gbolade A, Ekor MN, Akinlolu A, Ayoola MD. Antidiabetic activity of ethanolic extracts of *Spondias mombin* stem bark on alloxan-induced diabetic rats. J Pharmt Res 2008;7:192-5.
16. Gbolade AA, Akinlolu AA, Odewabi AO. Anti-hyperglycaemic and hypolipidemic activities of stem bark ethanol extract of *spondias mombin* l. (anacardiaceae). Niger Journ Pharm Sci 2011;10:42-51.
17. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001;50:537-46.
18. Oyedemi S, Bradley G, Afolayan A. Antidiabetic Activities of Aqueous Stem Bark Extract of Strychnos henningsii Gilg in Streptozotocin-nicotinamide Type 2 Diabetic Rats. Iran J Pharm Res 2012;11:221-8.
19. Adelaja AA, Ayoola MD, Otulana JO, Akinola OB, Olayiwola A, Ejiwunmi AB. Evaluation of the histo - gastroprotective and antimicrobial activities of *heliotropium indicum* linn (boraginaceae). Malays J Med Sci 2008;15:22-30.

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