Diabetogenic potential of dexamethasone and effect *Annona muricata* methanolic bark extract as post-exposure therapy in albino rats

¹Oladipo, T. A., ²Adeleye, O. E., ¹Olorode, J., ³Adebiyi, A. A., ⁴Omobowale, O. O., Adeoye, Y.D., ³Adenubi, O. T. Oladipo, O.O.⁵ and ^{2*}Olukunle, J. O.

¹Federal College of Animal Health and Production, Technology, Moor Plantation, Ibadan

²Federal University of Agriculture, Abeokuta, Department of Veterinary Physiology and Pharmacology, College of Veterinary Medicine

³Federal University of Agriculture, Abeokuta, Department of Veterinary Pathology, College of Veterinary Medicine

⁴University of Ibadan, Department of Veterinary Medicine, Faculty of Veterinary Medicine

⁵Federal College of Agriculture, Moor Plantation, Ibadan

Corresponding author: drfaks@yahoo.com; Phone: +234 810 184 6078

Abstract

Diabetes mellitus (DM) is a metabolic disorder leading to high level of morbidity and mortality in human population and it has been identified as the leading cause of death from non-infectious diseases. The use of dexamethasone has been on the increase due to wide array of therapeutic effects it has and the use has mostly been without prescription, since it is a non-prescription drug. Therefore, the potential of dexamethasone to induce DM was studied. Some of the drugs currently used in the treatment of diabetes have their own problematic effects and also expensive, *Annona muricata* methanolic bark extract (AMMBE) was used in the treatment of dexamethasone-induced diabetes in rats. The research was carried out on albino rats in Federal College of Animal Health and Production Technology, Ibadan and lasted for twenty-eight (28) days. The rats were allotted into four groups (A, B, C and D). Group B, C and D were induced with glucocorticoid (Dexamethasone) (2mgkg^{-1}) daily for seven (7) days intraperitoneally, while group A which was the positive control were given distilled water throughout without induction with glucocorticoid. Group B (the negative control) was induced with glucocorticoid with no AMMBE administration. Group C, was induced and treated with AMMBE at 400mgkg^{-1} for 14 days. While Group D, was induced with glucocorticoid and were treated with the standard drug (glibenclamide) at 2.5mgkg^{-1} body weight of the rats daily for 14 days. Organ samples of liver, kidney and pancreas were collected for histopathological lesions evaluation. The result showed that dexamethasone induced diabetes after seven (7) days of intraperitoneal administration of 2mgkg^{-1} body weights with the glucometer readings in most of the albino rats up to and above 129mgdL^{-1} . The average blood sugar levels in induced groups (B, C and D) were 132.0 ± 4.05 , 129.0 ± 1.41 and 130.0 ± 2.93 , respectively which were not statistically significant ($P > 0.05$). After administration of AMMBE, the average blood sugar level for group B (126.0 ± 1.41) was significantly different ($P < 0.05$) from C (91.0 ± 1.72) and D (87.0 ± 2.97). Clinical signs of alopecia, dehydration, writhing, paw-licking were observed. There were massive losses of pancreatic cell mass grossly after induction with dexamethasone. Histopathological lesions observed ranges from no visible lesion in the control and glibenclamide treated groups to accentuation of hepatocytes in the AMMBE treated rats, and marked vacuolar degeneration of hepatocytes in periportal areas to centrilobular area with Kidney degeneration and multifocal coagulation necrosis of tubular epithelium in group induced with dexamethasone but untreated (group B)

degeneration, multifoci coagulation and necrosis. The blood sugar levels post exposure to AMMBE and Glibenclamide showed reduction in the sugar levels. It could be concluded that dexamethasone has the potential of inducing diabetes when its use is prolonged and AMMBE has antidiabetic effect which could be fully explored.

Keywords: Dexamethasone, Diabetes mellitus, *Annona muricata*, Albino rats, Histopathology

Introduction

Diabetes mellitus (DM) is a metabolic disorder resulting from a defect in insulin secretion, insulin resistance, or both. Insulin deficiency leads to chronic hyperglycaemia with disorders of carbohydrate, fat and protein metabolism (Kumar and Clark, 2012). The disease leads to severe diabetic complications such as hepatopathy, nephropathy, retinopathy, neuropathy, ulceration etc as it progresses (Bearse *et al.*, 2004). Dogs and cats' family are mostly affected by the disease. Spontaneous diabetes in particular occurs in middle-aged dogs and middle-aged to older cats (David Bruyette, 2016). In dogs, females are affected twice as often as males and occurrence appears to be increased in certain small breeds such as Miniature Poodles, Dachshunds, Schnauzers, Cairn Terriers, and Beagles, but any breed can be affected (David Bruyette, 2016). In dogs, beta (β) cells reduction, insulin resistance following chronic administration of glucocorticoids (e.g dexamethasone) or progestins are seen. While cats with diabetes mellitus usually have specific degenerative lesions localized selectively in the islets of langerhans, in human subjects, it is estimated that more 300 million people will suffer from this disease by 2025 (Amos *et al.*, 1997; King *et al.*, 1998; Zimmet 2000). The inception for fasting glucose was changed from 7.8mmol/L (140 mg/dL) to 7.0 mmol/L (126 mg/dL) in man and this value has also been considered as diabetic even in rats (ADA, 2007)

Annona muricata also known as soursop or

graviola, is a member of the Annonaceae family and with long history of traditional use usually due to acetogenins it contains. A wide array of ethno-medicinal activities are attributable to different parts of *A. muricata* especially in Africa and South America. (Moghadamtousi *et al.*, 2015). Scientific investigations have validated its use as hypoglycemic, anticancer, antidiabetic interventions (Degnon *et al.*, 2013). Medications known as glucocorticoids, such as dexamethasone, prednisone and cortisone, are mainly used as anti-inflammatories or as anti-rejection drugs. They are prescribed, for example, for an arthritis attack or after an organ transplant. One of their side effects is the ability to increase blood glucose (sugar), since these drugs promote glucose production in the liver and reduce the sensitivity of the cells to insulin. Consequently, glucose accumulates in the blood and can cause a rise in blood sugar levels.

It has been reported that diabetes mellitus is currently the major cause of death from non-infectious disease all over the world therefore, any drug that has the likelihood to induce diabetes should be investigated and used with care. Also, there have been increasing need to discover a remedy to this disease from non-convention sources. Hence, the search of new anti-diabetic therapy can be fully justified by the increasing prevalence of types 1 and 2 diabetes and side-effects of current hypoglycemic drugs. This study therefore focusses on the determination of diabetogenic potential of dexamethasone in albino rats and the determination of *Annona*

muricata effectiveness as post exposure therapy to diabetes mellitus in albino rats.

Materials and methods

Experimental site

The experiment was conducted at the animal house, at the Federal College of Animal Health and Production Technology, Moor Plantation, Ibadan.

Experimental materials

The experimental material includes the following, *Annona muricata* bark, methanol, cotton wool, 5litres plastic with cover, plastic rubber cage, wood shaving, Conical flask, funnel, filter paper, muslin cloth, water bath apparatus, dissecting sets, hand glove, nose mask, needle and syringe (1mL and 2mL), Dexamethasone, Glibenclamide, glucometer, strips, sample bottles, water tank, digital weighing scale, transport basket, rat chow (feed) distilled water etc.

Preparation of extract and identification of species (AMMBE)

Fresh bark of *Annona Muricata* (soursop) was collected from the college vicinity and chopped into smaller size before it was sundried. Small sample of (AMBE) was taken to the Herbarium, Department of Botany, University of Ibadan, for identification of species.

The sundried *Annona muricata* bark were ground into powder. 500g of (AMBE) was soaked into 2 litres of methanol and left for 72 hours. The solution was squeezed and filtered with a muslin cloth and the filtrate was poured into conical flask to be concentrated on water bath at 40^o C until semi-solid extract was gotten with a yield of 29g, and then dissolved in DMSO before diluted into 100 mL Distilled water to give 290mg/mL concentration. The methanolic (AMMBE) extract was stored in a refrigerator prior use.

Experimental animal and duration of experiment

Apparently healthy male Albino rats (n=24) weighing 150 – 200g body weight were purchased from Research Animal World Laboratory (Rawlab), Physiology Department, University of Ibadan. The animals were acclimatized for 7days and fed on rat chow. The rats were acclimatized for seven (7) days, induced for 7 days and drenched/treated for 14 days. The experiment lasted for 28days.

Experimental design

The experiment rats were allotted into four (4) groups A, B, C and D with six (6) animals per group all fed on rat's chow, using completely Randomized design. Group B, C and D were induced with glucocorticoid (Dexamethasone) (2mgkg⁻¹) daily for seven (7) days intraperitoneally.

Group A which was the positive control were given distilled water throughout without induction with glucocorticoid. Group B, the negative control was induced with glucocorticoid and was not treated with methanolic extract of *Annona muricata* bark (AMBE). Group C, was induced and were treated with the methanolic extract of *Annona muricata* bark (AMBE) at 400 mgkg⁻¹ for 14days. Group D, was induced with glucocorticoid and were treated with the standard drug (glibenclamide) at 2.5 mgkg⁻¹ per body weight of the rats daily for 14 days. Group C and D were treated and were checked to determine the potential of the methanolic extract of *Annona muricata* bark (AMBE) over the standard drug (glibenclamide) as post exposure therapy of diabetic in the Albino rats.

Determination of diabetic status

Glucometer was used with strip and blood sample collected at the tail and readings were taken. Rat having sugar level of 126

mgdL⁻¹ and above were considered diabetic, while animals with sugar level above 70 mgdL⁻¹ were considered hyperglycaemic.

Organ samples harvested

Animals were sacrificed after sedation with cottonwool soaked with chloroform in a desiccator. The animals were dissected by opening up of the abdominal cavity and then, the following organs were collected; Group A – Kidney, liver and Pancreas, while in Group B, C and D Kidney and liver were harvested, because there were massive loss of pancreatic cell mass after the administration of glucocorticoid (dexamethasone) in Group B, C and D, which stimulation diabetes mellitus.

Laboratory analysis and results interpretation

The samples collected were preserved in 10% formalin and sealed up in sample bottles and were taken to the Department of Veterinary Pathology Laboratory, University of Ibadan, for histopathology. The histopathology results were interpreted

by a Veterinary Pathologist.

Results

The observable clinical signs in albino rats after induction with dexamethasone is shown in Table 1. There were no observable signs in rats on the control group that without treatment with dexamethasone. The group induced with dexamethasone but not administered with plant extract showed weight loss, condition characterized by ruffled hair coat, alopecia, writhing, paw-licking, dehydration and excessive thirst. There were Initial loss of condition with depraved appetite followed by restoration of skin condition and gradual rehydration in the group induced with dexamethasone and administered with 400mg/kg *A. muricata methanolic*. Whereas, there were rapid restoration of condition associated with increased appetite and water intake in the group of animals induced with dexamethasone and treated with glibenclamide (Table1).

Table 2: Average fasting blood sugar levels (mg/dL) of albino rats before, after induction with dexamethasone and post induction therapy (2 mgkg⁻¹)

Groups	Before induction (mgdl ⁻¹)	After induction (mgdl ⁻¹)	Post induction therapy (mgdl ⁻¹)
A (control)- not induced	26.0±2.10 ^{ab}	Not induced	36.0±2.09 ^d
B (dexamethasone -induced with no extract)	30.0±3.95 ^a	132.0±4.05 ^a	126.0±1.41 ^a
C (dexamethasone -induced treated with <i>A. muricata</i> extract)	28.0±4.97 ^{ab}	129.0±1.41 ^a	91.0±1.72 ^b
D (dexamethasone -induced with glibenclamide)	25.0±3.69 ^b	130.0±2.93 ^a	87.0±2.97 ^c

N/B- Means with different superscripts are statistically significant

Histopathology lesions observed at post exposure therapy

The histopathology of organs of rats in the control group is shown in Plate 1. The kidney shows normal histological

structures (glomeruli and tubules) and no observable lesion. The liver also shows normal histological structures (hepatocytes arranged in plates) and no observable lesion while the pancreas shows no observable lesion.

Plate 1: Histopathology of organs of rats in the control group (Islet (blue arrow) and acinar cells (red arrow) (HE x400)

The histopathology of organs of rats in the group induced with dexamethasone and not exposed to the plant extract is as seen in Plate 2. Liver in the plate, shows marked vacuolar degeneration of hepatocytes

(arrows) in periportal areas (zone 1) to centrilobular area (zone 3), while kidney shows degeneration and multifocal coagulation necrosis of tubular epithelium (arrows) (HE x400).

Plate 2: Histopathology of organs of rats in the group induced with no extract

The histopathology of organs of rats induced and treated with plant extract is shown in plate 3. Mild atrophy of hepatic

cords and accentuation of sinusoids is seen in the liver and no observable lesion is seen in the kidney (red arrow)

Plate 3: Histopathology of organs of rats induced and treated *Annona muricata* bark extract

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The histopathology of organs of rats induced and treated with glibenclamide is

shown in Plate 4. Liver and kidney have no observable lesions.

Plate 4: Histopathology of organs of rats induced and treated with glibenclamide

Discussion

The control group (not induced with dexamethasone) had no evidence of clinical signs exhibited in the rats, while group B which was induced but not treated presented with clinical signs like weight loss, change in condition characterized by ruffled hair coat, alopecia, writhing, paw-licking, dehydration, excessive thirst. This observation was similar to the findings of Vardy *et al.* (2006), who observed indigestion/epigastric discomfort, agitation, increased appetite, weight gain and acne (loss of body condition) in the week following chemotherapeutic use of dexamethasone. Groups C and D which were treated with AMMBE and glibenclamide, respectively had moderate to rapid restoration of body conditions, which was similar to the observation of Omotayo *et al.* (2011) that honey as an adjunct to glibenclamide or metformin improved glycemic control and some additional metabolic benefits not achieved with either glibenclamide or metformin alone.

Table 2 showed that the albino rats under study had normal blood glucose levels for groups A, B, C and D which were 26.0 ± 2.10 , 30.0 ± 3.95 , 28.0 ± 4.97 and

25.0 ± 3.69 , respectively before they were induced. But after the induction of groups B, C and D with dexamethasone there was development of high sugar levels that fell within the diabetic mellitus range (Type II) having fasting blood sugar levels of 132.0 ± 4.05 , 129.0 ± 1.41 and 130.0 ± 2.93 , respectively. This was in agreement with the work of John *et al.* (2008), that prolonged exposure to elevated glucocorticoid levels is known to produce insulin resistance (IR), a hallmark of type 2 diabetes mellitus. The use of AMMBE and glibenclamide as post induction therapy led to reduction in the fast blood glucose levels when compared to the diabetic untreated group. This further affirmed the work of Degnon *et al.* (2013), that different parts of *Annona muricata* has antidiabetic effects.

The massive loss of pancreatic cells was eminent in the induced groups but not seen in the control group as observed in the histopathological findings. In the diabetic untreated group (group B) there were marked vacuolar degeneration of hepatocytes in periportal areas to centrilobular area in the liver with degeneration and multifocal coagulation necrosis of tubular epithelium in the kidney. Also, in diabetic induced group treated with

AMMBE (group C), there were mild atrophy of the hepatic cords and accentuation of the sinusoids in the liver, but with no observable lesions in the kidney. While in the glibenclamide treated group (group D) there were no observable lesions in the liver and kidney of the albino rats.

Conclusion

From this study, it could be concluded that the use of glucocorticoids in albino rats led to the establishment of persistent high blood sugar levels and diabetes in groups B, C and D, after seven (7) days of administration of 2mgkg^{-1} . There were massive losses of pancreatic cells after the administration of glucocorticoids, simulating type I diabetes mellitus. The use of *Annona muricata* bark extract led to gradual reduction in the blood sugar level, but with slight lesions on the hepatocytes at histopathology. Also, *Annona muricata* bark extract when compared with standard antidiabetic drug showed a great potential in the treatment of diabetes mellitus.

Recommendations

It is therefore recommended that further studies be done on *Annona muricata* methanolic bark extract as a potential agent in the treatment of diabetes mellitus.

References

- Amos, A., McCarty, D. and Zimmet, P. 2010.** The rising global burden of diabetes and its 9 complications, estimates and projections to the year 2010. *Diabetic Medicine* 14: S1-S85.
- Bearse, M. A Jr., Han, T. and Schneck, M. E. 2004.** Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. *Investigation Ophthalmic Vision Science* 45: 3259-3265.
- David, B. 2016.** Merck and the Merck Veterinary Manual *Merck and Co., Inc., Kenilworth, NJ, USA*
- Degnon, R. G., Adjou, E. S., Noudogbessi, J. P., Metome, G., Boko, F., Dahouenon-Ahoussi, E., Soumanou, M. and Sohounhloue, D. C. K. 2013.** Investigation on nutritional potential of soursoup (*Annona muricata* L.) for its use as food supplement against protein deficiency. *Intenational Journal of Biosciences* 3 (6): 135-144.
- John, S. G., Marion, K., Karen, K., Gregory, A., Oliver, T. and Didier, L. 2008.** Effect of Dexamethasone on Glucose Tolerance and Fat Metabolism in a Diet-Induced Obesity Mouse Model. *Endocrinology*, Volume 149, Issue 2, 1 February, Pages 758-766,
- Kumar, P. J., Clark, M. 2002.** Textbook of Clinical Medicine. Pub: Saunders (London), pp 1099-1121,
- Moghadamtousi, S. Z., Fadaeinasab, M., Nikzad, S., Mohan, G., Ali, H. M. and Kadir, H. A. 2015.** *Annona muricata* (Annonaceae): A Review of Its Traditional Uses, Isolated Acetogenins and Biological Activities. *International Journal of Molecular Science*. Jul 10;16 (7) : 1 5 6 2 5 - 5 8 . doi : 10.3390/ijms160715625.
- Siti, A. S., Mohd, S., Wahab, A. B., Kuttulebbai, N., Salam, S. 2011.** Glibenclamide or Metformin Combined with Honey Improves Glycemic Control in Streptozotocin-Induced Diabetic Rats. *International Journal of Biological Science*. 7(2): 244-252.
- Vardy, J., Chiew, K. S., Galica, J., Pond,**

G. R., Tannock, I. F. 2006. Side effects associated with the use of dexamethasone for prophylaxis of delayed emesis after moderately emetogenic chemotherapy. *British Journal of Cancer*: Apr 10; 94(7): 1011–1015.

Zimmet, P. 2004. Globalization, coca-colonization and the chronic disease epidemic: can the Doomsday scenario be averted? *Journal of Medicine* 247: 301-310.

Received: 12th July, 2018

Accepted: 9th February, 2019