British Journal of Pharmaceutical Research
6(5): 333-342, 2015, Article no.BJPR.2015.075
ISSN: 2231-2919

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antidiabetic Activity and Toxicity Evaluation of Aqueous Extracts of *Spondias mombin* and *Costus afer* on Wistar Rats

Moke Emuesiri Goodies[1*], Ilodigwe Emeka Emmanuel[1], Okonta Jemefuna Matthew[2], Emudainohwo Joseph Oghenebrorie Tedwins[3], Ajaghaku Daniel Lotanna[1], Erhirhie Oghenesuvwe Earnest[1], Chinwuba Paul[1] and Ahante Ejiroghene[4]

[1]Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Agulu Campus, Anambra State, Nigeria.
[2]Department of Clinical Pharmacy, University of Nigeria, Nsukka, Enugu State, Nigeria.
[3]Department of Pharmacology and Therapeutics, Delta State University, Abraka, Delta State, Nigeria.
[4]Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, Enugu State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors MEG, IEE and OJM designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors MEG, IEE, ADL and EOE managed the literature searches, analyses of the study performed the spectroscopy analysis and authors MEG, EJOT, EOE, AE and CP managed the experimental process and author AE identified the species of plant. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJPR/2015/17519
Editor(s):
(1) Jinyong Peng, College of Pharmacy, Dalian Medical University, Dalian, China.
Reviewers:
(1) Anonymous, Turkey.
(2) Anonymous, Belgium.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=986&id=14&aid=8846

Original Research Article

Received 17th March 2015
Accepted 1st April 2015
Published 16th April 2015

ABSTRACT

Aims: To evaluate the antidiabetic efficacy of the aqueous extracts of *Spondias mombin* leaf and *Costus afer* stem, both individually and in combination ratios, as well as the acute and subchronic toxicities of the most effective antidiabetic combination ratio.
Study Design: Antidiabetic study was carried using diabetic animals grouped into seven of five animals each.
Place and Duration of Study: Department of Pharmacology and Toxicology, Nnamdi Azikiwe

**Corresponding author: Email: hiligoodies@gmail.com, hiligoodies@operamail.com;*

University, Awka, Anambra State, Nigeria, between May 2013 and June 2014.
Methodology: Alloxan-induced diabetic rats received 100, 200 and 400 mg/kg of the extracts orally; control groups received glibenclamide (5 mg/kg, oral) and normal saline. Blood glucose was measured 2-hourly for 6 hours and weekly for 28 days. The LD_{50} of both plants was estimated using the modified Lorke's method.
Results: The toxicity of the 1:1 mixture on body weight, haematological indices (RBC, WBC, PCV, and haemoglobin), and liver enzymes (AST, ALT, and ALP) were assessed for 60 days. The aqueous extracts of *Spondias mombin* and *Costus afer* showed significant ($p>0.05$) hypoglycaemic effect, with the 1:1 ratio having the best antidiabetic effects among the ratios when co-administered. There were non-significant ($p>0.05$) variations in weight change, RBC, WBC, PCV, and hemoglobin concentration, as well as in AST, ALT, and ALP levels of the test groups.
Conclusion: This study justifies the antidiabetic activity of the aqueous extracts of *Spondias mombin* and *Costus afer*, both individually and as mixtures of various ratios, especially when combined in equal proportions (1:1).

Keywords: Spondias mombin; *Costus afer; alloxan; herbal decoction; diabetes; toxicity.*

1. INTRODUCTION

Diabetes is a disease associated with glucose metabolism resulting from defects in insulin secretion and action [1]. It is recognized as one of the leading causes of mortality and morbidity in the world [2]. The prevalence of diabetes in Nigeria with over 250 tribes was recorded by the International Diabetes Federation as 3.9% [3].

Despite the numerous antidiabetic drugs, natural products still remain the treatment of choice especially in developing countries and rural settlements. Natural products use in the management of diabetes is attributed to their effectiveness, less side effects and relatively low cost [4]. Natural products from medicinal plants such as; *Gynura bicolor* [5], *Thuamatococus daneilli* [6] among others [7], had been scientifically proven to posses hypoglycemic properties.

Spondias mombin (Anacardiaceae) is a plant whose leaf, bark, roots and seeds are used for medicinal purposes. The ethanol extract of the stem bark have been shown to possess antidiabetic activity on rats [8]. Fred-Jaiyesimi et al. [9] had also identified antidiabetic principle (3β-olean-12-en-3-yl (9*Z*)-hexadec-9-enoate, an α- amylase inhibitor, isolated from *S. mombin* leaf as an alkane derivative.

Costus afer (Costaceae) is a perennial and rhizomatous herb found in Nigeria and commonly called bush cane [10]. The aqueous leaf and stem bark extracts of *Costus afer* are being used in the treatment of diabetes mellitus in folklore medicine [11]. Its stem extract has also been shown to have antioxidant effect [12].

In the rural communities of Delta State, various combination ratios of the leaf of *Spondias mombin* and stem of *Costus afer* decoctions have been widely used for the control of diabetes. Despite the popular use of these decoctions, no information exists about the most effective ratio. Also, since diabetes is a chronic disorder, this study evaluated the antidiabetic effectiveness of both plant parts, the most effective combination ratio, and the subchronic toxicities of the most effective ratio.

2. METHODOLOGY

2.1 Animals

Wistar rats of both sexes weighing between 120-240 g were obtained from the colony breed of the animal house of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu, Anambra State. The rats were kept in cages under standard conditions and fed with standard diet (Growers feed) and clean water *ad libitum*. The animals were allowed to acclimatization for two weeks prior to the commencement of the experiment. Animals were handled in compliance with NIH Guide for care and use of laboratory animals (pub. No. 85-23 revised 1985), and approved by the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University's ethical committee for the use of laboratory animals.

2.2 Plant Material

Fresh leaves of *Spondias mombin* and stems of *Costus afer* were collected from the school garden of Delta State University, Abraka and

authenticated by the Department of Pharmacognosy, Faculty of Pharmacy, Delta State University, Abraka.

2.3 Plant Extraction

The plant materials were cut into pieces, air-dried at ambient temperature and blended into coarse powder. The powdered *Spondias mombin* (500 g) was dissolved in 1500 ml of distilled water, while the powdered *Costus afer* (500 g) was mixed in 2500 ml of distilled water. Both suspension were stirred every six hours. After 72 hours the solutions were then filtered and the filtrate concentrated to dryness using a freeze-dryer to yield a residue, 49.6 g (9.9%) for *Spondias mombin* and 18.2 g (3.6%) for *Costus afer*.

2.4 Acute Toxicity (LD_{50})

The acute toxicity (LD_{50}) study was carried on *Spondias mombin* and *Costus afer* using a modified Lorke's method [13]. Animals (rats and mice) of either sex were fasted overnight prior to the study. Oral doses of 10, 100, 1000 mg/kg were used in phase I, while 2000, 3000, 4000, and 5000 mg/kg were given in phase II to the treated groups; the control groups received 10 ml/kg of normal saline. The median lethal dose (LD_{50}) was estimated after observing the animals for 24 hours for obvious toxic symptoms or mortality.

2.5 Antidiabetic Studies

Animals were fasted for 18 h (but allowed access to water) before induction of hyperglycaemia, which was accomplished by intraperitoneal injection of 150 mg/kg of alloxan monohydrate [8]. Animals were fed *ad libitum* 1 h after alloxanisation. Fasting blood glucose (FBG) was monitored before and after alloxanisation from samples collected from the tail tip, by means of a glucometer (ACCU-CHEK® Active) and compatible blood glucose test strips. The diabetic state of the animals was assessed by FBG measurement 72 h after alloxanization, and rats with FBG of ≥ 150 mg/dl were selected for the study [14].

2.6 Experimental Design

The diabetic animals were divided into seven groups; Group 1 received 10 ml/kg of normal saline, Group 2 received glibenclamide 5 mg/kg, Groups 3 and 4 received 100 and 200 mg/kg of the individual plant extracts, while Groups 5-7 received combined extracts of *Spondias mombin* and *Costus afer* at the doses of 100, 200, and 400 mg/kg in the ratios of 1:1. 1:2 and 2:1 respectively.

After a single oral administration of the extracts, blood from the rat's tail vein was obtained hourly for 6 hours and the blood glucose determined using a glucometer. Thereafter, daily administration of the extracts continued for 4 weeks and blood glucose measured every week. The percentage change in blood glucose, presented in parenthesis, was calculated using the formula below.

$$\% \text{ Blood Glucose} = \frac{(G_{initial} - G_{final})}{G_{initial}} \times 100$$

Where $G_{initial}$ is initial blood glucose level and G_{final} is final blood glucose level.

2.7 Toxicity Studies

Haematological and liver toxicities were carried out for 2 months using forty rats which were randomly divided into four groups of 10 rats each. After pretreatment studies, Group 1 served as control and received normal saline (orally). Groups 2-4 received 250 mg/kg, 500 and 1000 mg/kg of oral doses of the combined aqueous extracts of *Spondias mombin* and *Costus afer* (1:1) respectively. Toxic manifestations and mortality were monitored daily and the body weight changes were also recorded every 7 days.

Blood samples were withdrawn from all groups of animals at the 31 and 61st days, and analyzed for toxicological parameters relating to liver function (aspartate aminotransferase, AST, alanine aminotransferase, ALT, and alkaline phosphate, ALP), and haematological indices (packed cell volume, PCV, haemoglobin concentration, Hb, red blood cell count RBC, and white blood cell count, WBC). After blood withdrawal, animals were sacrificed and the livers from two rats per group were harvested for histopathological analysis [15].

2.8 Statistical Analyses

Significant difference between control and experimental groups were obtained by student's t-test using SPSS version 16. All data obtained were expressed as Mean±SEM (standard error

of mean). Graphical representation was done using Microsoft Excel 2010. P-values <0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Acute Toxicity (LD_{50})

No death was recorded in the acute toxicity testing for all animals and there was also no obvious signs of toxicity in all treatment groups in both species (rat and mice) and phases (1 and 2) when observed within 24 hours of post-administration of the aqueous extracts of *Spondias mombin* and *Costus afer*. The LD_{50} of the extracts was greater than 5000 mg extract/kg body weight.

3.2 Effects on Blood Glucose

Figs. 1-3 show the effects of the plant extracts and the various combination ratios on blood glucose levels in diabetic rats.

Following the first 6 hours of initial treatment, both *Spondias mombin* (SM) treated groups and *Costus afer* (Costus) treated group had 18.78 and 18.55% reduction in FBS at 100 mg/kg, 8.44 and 12.01% FBS reduction at 200 mg/kg, and 16.15 and 24.30% FBS reduction at 400 mg/kg respectively. It was seen that at 400 mg/kg, Costus reduction of blood glucose was as high as GLB (glibenclamide) which was 24.64%. The blood glucose in the untreated diabetic group reduced by 4% (288±33.47 to 276.2±31.62 mg/dl). This showed that Costus had a higher hypoglycaemic effect than *Spondias mombin* with single dosing. The percentage reduction of blood glucose by the combination ratios of *Spondias mombin* to Costus, 1:1, 1:2, and 2:1 showed 10.79, 10.20, and 7.56% at 100 mg/kg, 13.16, 3.24, and 8.26% at 200 mg/kg, and 13.93, 9.23, and 9.73% at 400 mg/kg respectively after 6 hours of single dosing. All ratios produced a weak hypoglycaemic activity (3.24-13.93%), with 1:1 ratio having the best activity and 1:2 ratio having the least activity.

Weekly determination of FBS level showed that by the fourth week, the blood glucose was significantly (P<0.05) decreased in the treated groups, whereas there was an increase in blood glucose level in the untreated diabetic group. In the *Spondias mombin* treated group, the blood glucose significantly decreased by 60.87, 64.29, and 61.10% at 100, 200, and 400 mg/kg respectively after 4 weeks, while the Costus treated group showed a significant decrease in FBS by 44.91, 38.70, and 53.63% at 100, 200, and 400 mg/kg respectively. The glibenclamide-treated group showed a 63.78% blood glucose reduction. In contrast, the untreated diabetic group had an increase in blood glucose from 288±33.47 to 314.4±39.91mg/dl (9.17%). This indicates that *Spondias mombin* has a higher activity in lowering blood glucose than Costus. *Spondias mombin* had the highest hypoglycaemic activity at 200 mg/kg, (64.29%), even better than the glibenclamide-treated group. Evaluation of the hypoglycaemic activity of the different ratios of the mixture of both *Spondias mombin* and Costus extracts showed that 1:1, 1:2, and 2:1 reduced blood glucose by 55.65, 54.12, and 54.37% respectively at 100 mg/kg, 56.68, 47.14, and 47.26% at 200 mg/kg, and 57.04, 43.86, and 56.07% at 400 mg/kg after 4 weeks of treatment. Ratio 1:1 of the mixture showed the best activity when administered at 100, 200 and 400 mg/kg, whereas the 1:2 ratio showed the least hypoglycaemic activity (43.86%) among all three mixtures.

3.3 Subchronic Toxicity Study

In the toxicological study, 250, 500, and 1000 mg/kg of oral doses of the combined aqueous extracts of *Spondias mombin* and *Costus afer* (1:1) were administered to animals.

3.3.1 Effects on body weight

There was insignificant (p>0.05) increase in body weight of the animals when compared to the control (Table 1).

3.3.2 Effects on haematological indices

A non-significant difference (p>0.05) in hematological indices such as packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC) count, and haemoglobin (Hb) concentration was observed between control and treated groups during the two months duration (Tables 2 and 3).

3.3.3 Effects on liver enzymes

The treated groups showed insignificant (p>0.05) changes in serum levels of ALT, AST and ALP when compared with the control group (Tables 4 and 5).

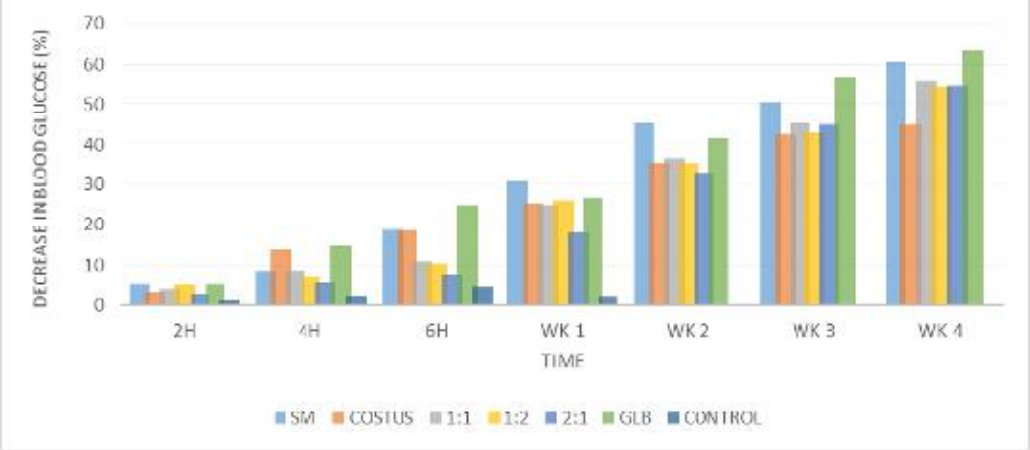

Fig. 1. The effect of 100 mg/kg of plant extracts on blood glucose level of alloxan-induced diabetic rats

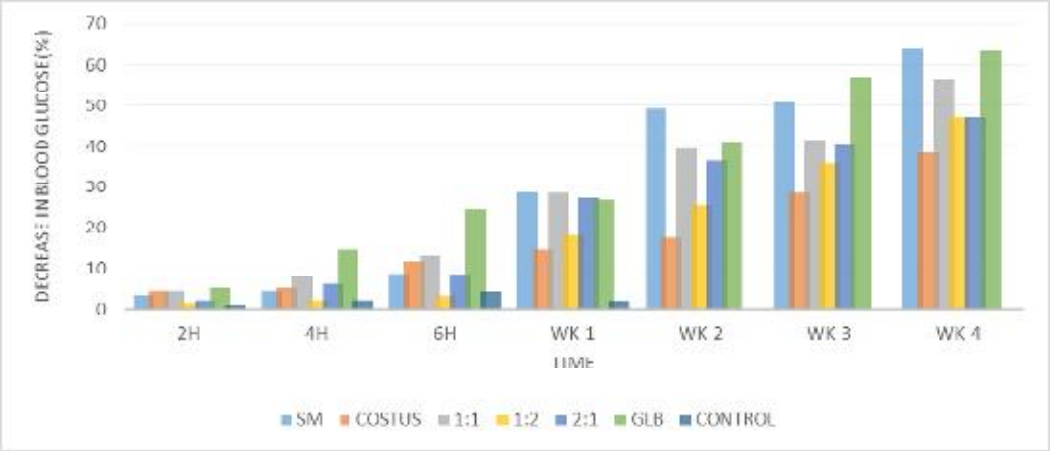

Fig. 2. The effect of 200 mg/kg of plant extracts on blood glucose level of alloxan-induced diabetic rats

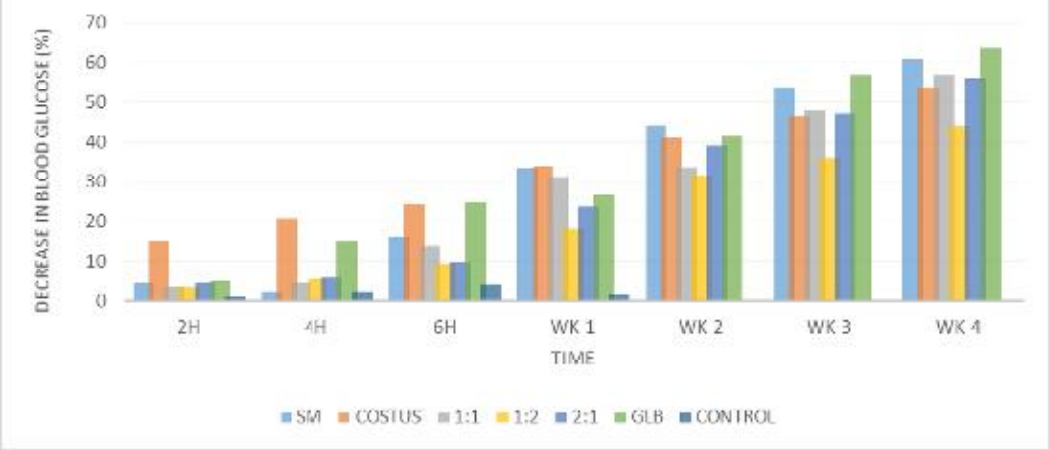

Fig. 3. The effect of 400mg/kg of plant extracts on blood glucose level of alloxan-induced diabetic rats

3.3.4 Histopathology of the liver

At the end of sub-chronic study, photomicrographs of the liver tissues showed mild to moderate infiltration by leucocytes on hepatocytes and mild hepatocellular necrosis in the treated groups (Fig. 4).

In this study, Type II diabetic animals that received herbal decoction of *Spondias mombin* (SM) and *Costus afer* (Costus) mixture at equal proportion (1:1) achieved antidiabetic activity than those that received the mixture at unequal ratios (1:2 or 2:1) of the extracts. There were no obvious adverse effects associated with receiving *Spondias mombin* and *Costus afer* mixture for the treatment of diabetes mellitus. The LD_{50} of the extracts was greater than 5000 mg extract/kg body weight.

From this study, it was observed that the administration of the aqueous extracts of *Spondias mombin* and *Costus afer* at different doses to diabetic rats resulted in significant ($p<0.05$) reduction of their plasma glucose levels. Following chronic usage (28 days), *Spondias mombin* was better of both extracts, in the reduction of the blood glucose. Although, for single dosing (acute use) *Costus afer* at 400 mg/kg is preferred since it effectively reduced hyperglycaemia than *Spondias mombin*. The combination of both *Spondias mombin* and *Costus afer* in the ratio of 1:1 yielded the best antidiabetic activity when used both acutely and over a prolonged period of time as compared with the 1:2 or 2:1. Neither 1:2 nor 2:1 ratio showed any better hypoglycaemic effect than the 1:1 ratio but they both reduced blood glucose by at least half after chronic administration especially at 100 and 400 mg/kg. This indicates that all mixtures were effective in lowering blood glucose.

The comparison between the hypoglycaemic activity of the individual plant parts and their mixtures following weekly evaluation revealed that *Spondias mombin* at 100, 200 and 400 mg/kg is better than both *Costus afer* and the plant parts mixtures (1:1, 1:2, and 2:1). Also, the 1:1 mixture produced a higher anti-hyperglycaemic activity than *Costus afer*. This implies that a better antidiabetic activity of their mixtures is attained when both extracts are combined in equal proportions.

Previous studies had assessed the antidiabetic potential of these individual plant parts. A study carried out by [16] showed that the methanol leaf extract of *Spondias mombin* and its chloroform fraction exhibited significant blood glucose lowering effect in experimental diabetic rats after 8 hours of administration when compared with the control. The chloroform fraction of *Spondias mombin* showed a peak hypoglycaemic effect of 60.6% at 4th hour. In another study, Nwauche and co-worker [17] validated the use of aqueous stem extract of *Costus afer* to treat streptozotocin-induced diabetic rats. At 500 mg/kg, blood glucose level remarkably decreased between the 3rd and 9th weeks of treatment.

The results of this present study is consistent with the findings of Fred-Jaiyesimi and Abo; as well as Nwauche and co-worker [16,17] where both individual plant parts of *Spondias mombin* and *Costus afer* produced significant ($P<0.05$) decline in plasma glucose level.

The animals treated with the extract mixture (1:1) showed increased appetite, food and water consumption, but there was non-significant difference ($P>0.05$) in their body weight gain (Table 1). This is an indication that the mixture is unlikely to cause obesity since the feeding patterns of the animals were normal [18].

Assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compounds, including plant extracts, on the blood constituents of an animal [19]. In this study, non-significant differences in the haematological indices (PCV, RBC, WBC, Hb concentration; (Tables 2-3) when compared to their respective control groups, suggest the unlikelihood of the aqueous extracts mixture of *Spondias mombin* and *Costus afer* (1:1) to induce anaemia or disrupt the circulating cells of the immune system on long term use [18,19,20,21].

Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) are largely used in the assessment of liver damage by drugs or other hepatotoxins. Liver cell permeability, congestion or cell rupture are characterized by a rise in serum enzymes like AST, ALT and alkaline phosphatase (ALP) [22]. The non-significant ($P>0.05$) increase in AST level and ALP, as well as the non-significant ($P>0.05$) decrease in ALT level on the 31 and 61st days (Tables 4 and 5) indicates that the aqueous extracts mixture of *Spondias mombin* and *Costus afer* (1:1) is less likely to cause hepatocellular damage or

cholestasis [23]. The photomicrographs of the liver revealed mild infiltration by leukocytes in treated groups that received 250, 500 and 1000 mg/kg of the 1:1 mixture for 60 days (Fig. 4).

Although, we are much encouraged with the apparent effects of the 1:1 decoction of *Spondias mombin* and *Costus afer*, further studies on the neprotoxic, mutagenic, and teratogenic effects on Wistar rats is needed to fully characterize the toxicity of this decoction.

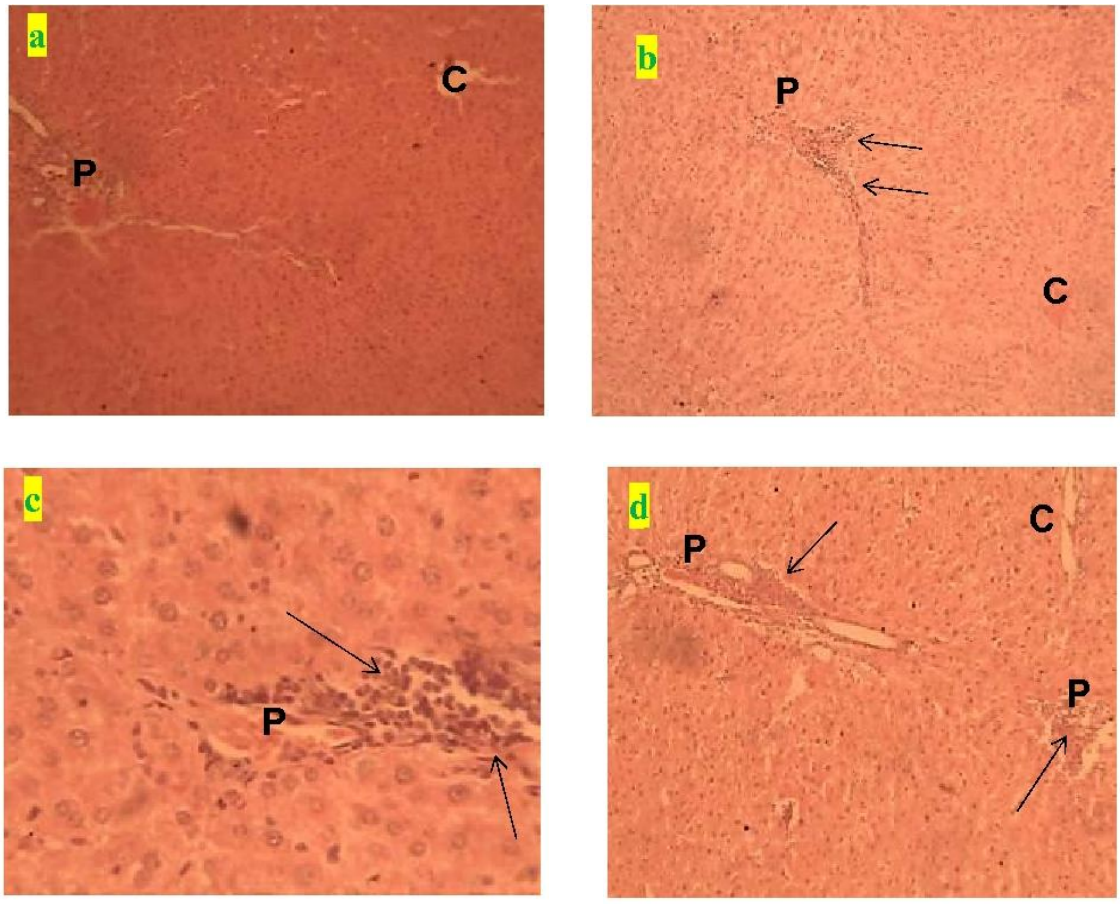

Fig. 4. Liver histology

(a) Control - Normal hepatocytes arranged in radiating chords around the central veins, (b) 250 mg/kg - Mild to moderate periportal infiltration of mononuclear leukocytes (arrow) with mild coagulative hepatocellular necrosis, (c) 500 mg/kg - Moderate infiltration of inflammatory leukocytes (arrow) around the portal area, (d) 1000 mg/kg - Moderate periportal infiltration of mononuclear leukocytes (arrow). P- Portal area, C- central vein

Table 1. Effects on body weight changes (g)

Dose (mg/kg)	Pre-treatment	31st day	61st day
250	113.02±4.38	160.96±9.58	198.12±10.97
500	122.64±9.41	189.34±11.92	245.62±15.02
1000	125.38±9.25	180.34±9.13	211.66±13.00
Control	138.50±10.65	184.46±8.47	210.46±5.25

Values are expressed as Mean ±SEM of sample replicates (n=5)

Table 2. Effect of *Spondias mombin* and *Costus afer* (1:1) on haematological indices of wistar rats treated for 30 days

Treatment	PCV (%)	RBC (10^3/mm^3)	WBC (10^3/mm^3)	Hb (g/dl)
250 mg/kg	45.00±2.26	43.32±4.37	24.84±1.03	11.26±0.47
500 mg/kg	42.00±2.29	42.80±4.85	24.02±1.58	12.94±0.82
1000 mg/kg	44.60±1.86	30.94±2.07	22.72±0.38	13.00±0.81
Control (normal saline, 10 ml/kg)	40.80±2.56	38.98±4.46	19.52±2.88	10.90±1.39

Values are expressed as Mean ±SEM of sample replicates (n=5)

Table 3. Effect of *Spondias mombin* and *Costus afer* (1:1) on haematological indices of wistar rats treated for 60 days

Treatment	PCV (%)	RBC (10^3/mm^3)	WBC (10^3/mm^3)	Hb (g/dl)
250 mg/kg	41.60±1.69	42.08±2.95	27.84±2.91	8.19±2.60
500 mg/kg	40.20±2.37	42.80±3.45	29.06±1.57	8.22±1.47
1000 mg/kg	35.80±2.01	32.90±1.43	31.54±0.86	5.48±0.57
Control (normal saline, 10 ml/kg)	39.40±3.59	39.04±2.99	31.42±3.33	5.23±0.94

Values are expressed as Mean ±SEM of sample replicates (n=5)

Table 4. Effect of *Spondias mombin* and *Costus afer* (1:1) on liver enzymes of wistar rats treated for 30 days

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
250 mg/kg	50.80±12.04	16.10±1.85	35.66±5.23
500 mg/kg	43.40±10.25	16.60±3.97	39.98±2.39
1000 mg/kg	49.20±12.97	16.40±1.91	52.60±5.19
Control (normal saline, 10 ml/kg)	60.80±10.63	22.70±2.53	41.42±3.01

Values are expressed as Mean ±SEM of sample replicates (n=5)

Table 5. Effect of *Spondias mombin* and *Costus afer* (1:1) on liver enzymes of wistar rats treated for 60 days

Treatment	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
250 mg/kg	39.80±13.70	11.30±3.60	34.98±3.32
500 mg/kg	40.60±13.55	9.10±2.37	34.78±4.65
1000 mg/kg	45.40±13.72	12.40±3.48	36.06±2.26
Control (normal saline, 10 ml/kg)	39.80±12.17	8.30±2.00	42.88±2.96

Values are expressed as Mean ±SEM of sample replicates (n=5)

4. CONCLUSION

This study has shown that the acute and sub-chronic (4 weeks) administration of the aqueous extracts of *Spondias mombin* and *Costus afer*, both individually and as mixtures of various ratios, resulted in effective anti-hyperglycemic activity in alloxanized rats; however, the result showed that the combination of both plants provide the best antidiabetic effect when they are of equal ratios (1:1). The study also showed that the 1:1 mixture is relatively safe at the doses tested. However, since diabetes is a chronic disease, liver toxicities should be intermittently evaluated when in use.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-

23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were examined and approved by the Nnamdi Azikiwe University's ethical committee for the use of Laboratory animals.

ACKNOWLEDGEMENTS

Special thanks to the Tertiary Educational Trust Fund (TETF) academic staff training and development committee, Delta State University, Abraka for funding this project, and also to Ahatty Godwin Cliford, and Mbagwu Ikechukwu Sonne for their laboratory assistance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. World Health Organization (WHO). Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1: Diagnosis and Classification of Diabetes. WHO/NCD/NCS 99, 2, Geneva, 1999;1-58.
2. Can A, Akev N, Ozsoy N, Bolkent S, Arda BP, Yanardag R, and Okyar A. Effect of *Aloe vera* leaf gel and pulp extract on the liver in type II diabetic rats models. Biol. Pharm. Bull. 2004;27:694-698.
3. Akogu I. The prevalence of diabetes mellitus in Nigeria updates and challenges; 2010 [cited 2014 Dec 22]. Available:http://diabetesguidenigeria.blogspot.com
4. Calixto JB. Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicine (Phytotherapeutic agents). Brazilian Journal of Medicine and Biological Research. 2000;33:179-189.
5. Jian CU, Sven M, Adams AN, Li WL, Wang ZT, De Kimpe N. Chemical constituents from the aerial parts of Gynura bicolor. Natural Product Commun. 2012;7(12): 1563-1564.
6. Emudainohwo JOT, Erhirhie EO, Moke EG, Ejebe DE. Hypoglycemic Effect of Ethanol Leaf Extract of *Thuamatococus daneilli* (ELETD) In Alloxan Induced Diabetic Wistar Rats. IOSR Journal of Pharmacy and Biological Sciences. 2015; 10(2):59-64.
7. Li WL, Zheng HC, Bukuru J, De Kimpe N. Natural medicines used in the traditional Chinese medical system for therapy of diabetes mellitus. J Ethnopharmacol. 2004; 92(1):1-21.
8. Gbolade AA, Ekor MN, Akinlolu AA, Ayoola MD. Antidiabetic activity of stem bark ethanolic extracts of *Spondias mombin* on alloxan-induced diabetic rats. J. Pharmaceut. Res. 2008;7:192-195.
9. Fred-Jaiyesimi A, Abo K. and Wilkins R. α-Amylase inhibitory effect of 3β-olean-12-en-3-yl (9Z)-hexadec-9-enoate isolated from *Spondias mombin* leaf. Food Chem. 2009;116:285-288.
10. Edeoga HO, Okoli BE. Chromosome numbers of *Costus lucanusianus* (Costaceae) in Nigeria. Folia Geobotanica. 2000;35:315-318.
11. Udem SC, Ezeasor CK. The acute and subchronic toxicity studies of aqueous leaf and stem bark extract of *Costus afer* ker (zingiberaceae) in mice. Comp Clin Pathol. 2010;19:75-80
12. Anyasor GN, Ogunwenmo KO, Oyelana AO, Akpofunure EB. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae). African Journal of Biotechnology. 2010; 9(31):4880-4884.
13. Lorke DA. A new approach to practical acute toxicity testing. Arch Toxicol. 1983;54:275-287.
14. Etuk EU. Animals models for studying diabetes mellitus. Agric Biol J N Am. 2010; 1:130-134.
15. Kieman JK. Histology and Histological Methods, Theory and Practice. 2nd Ed. Pergamon press, Exeter; 1990.
16. Fred-Jaiyesimi A, Abo K. Antidiabetic Activity of *Spondias mombin* Extract in NIDDM Rats. Pharmaceutical Biology. 2009;47(3): 215-218.
17. Nwauche KT, Monago CC, Anacletus FC. Antihyperglycemic activity of the aqueous extract of *Costus afer* stem alone and in combination with metformin. European Journal of Biotechnology and Bioscience. 2014;1(5):19-25
18. Obi HI, Ilodigwe EE, Ajaghaku DL, Okonta JM. An evaluation of acute and subchronic toxicities of a Nigerian polyherbal antidiabetic remedy. Int J Pharm Sci Res. 2012;3(9):3131-3135.
19. Ashafa AOT, Yakubu MT, Grierson DS, Afolayan AJ. Effects of aqueous leaf

extract from the leaves of *Chrysocoma ciliate L*. on some biochemical parameters of wistar rats. African Journal of Biotechnology. 2009;8:1425-1430

20. Cole EH. Veterinary Clinical Pathology, W. B Saunders. 1986;10-42.
21. Ochei J, Kolhatkar J. Medical laboratory science. Theory and Practice, 1st ed. McGraw Hill Medical; 2008.
22. Tedong L, Dzeufiet PD, Dimo T, Asongalem EA, Sokeng SN, Flejoy J, Callard P, Kamtchouing P. Acute and subchronic toxicity of *Anacardium occidentale* Linn (Anacardiaceae) leaves hexane extract in mice. African Journal of Traditional, Complementary and Alternative Medicines. 2008;4(2):140-147.
23. Nelson DL, Cox MM. Principles of Biochemistry. 4th ed. W.H. Freeman and Company, New York; 2005.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=986&id=14&aid=8846