Antidiabetic and Toxicity Studies of the Extract of Four Nigerian Medicinal Plants

ABSTRACT

Aims: To evaluate the anti-hyperglycaemic efficacy and safety of the methanol extract of the combination of *Senecio biafrae* leaf, *Xylopia aethiopica* fruit, *Carica papaya* seed and *Spondias mombin* stem bark mixed together in ratio 1:1:1:1

Study Design: The extract of medicinal plants was assayed using glucose and streptozotocin-induced hyperglycaemic rats model.

Place and Duration of Study: Department of Pharmacognosy, Obafemi Awolowo University, IleIfe, Nigeria, between May, 2019 and January, 2022.

Methodology: The extract of the combined plant parts was tested for toxicity in rats while its effects on glucose level, blood and biochemical components were also assessed. Its *in-vitro* anti-hyperglycaemic activity was assayed in α-amylase and α-glucosidase inhibitory models while its *in-vivo* effects were tested in glucose and streptozotocin-induced hyperglycaemic rats. The antioxidant activity of the extract was also carried out.

Results: The extract did not show any adverse effects on blood sugar levels, haematological and biochemical parameters in sub acute toxicity studies in normal rats. The extract gave comparable ($p > 0.05$) *in vitro* α-amylase and α-glucosidase inhibitory effects to acarbose. In *in vivo* glucose-induced hyperglycaemic rats, its 100 mg/kg was the most effective dose with 19, 40, 43, and 57 % activity that was significantly higher ($p < 0.05$) than the 10, 18, 24, and 40 % activity given by glibenclamide (5 mg/kg) at the same time points. In streptozotocin-induced diabetic assay, its 50 mg/kg showed 31, 85, 85 and 82 % effects on days 4, 7, 10 and 14, respectively that was significantly higher than its 100 mg/kg and glibenclamide on days 7 and 10. The extract also elicited high free radical scavenging effects in all the antioxidant assays.

Conclusion: The extract of the combination of four Nigerian antidiabetic plants mixed together in equal ratio gave significantly better antidiabetic activity at low doses than the individual component plants without toxic effects.

Keywords: Diabetes mellitus, anti-hyperglycaemic effect, plant combination, antioxidant activity.

1.0. INTRODUCTION

Diabetes mellitus is a collection of severe metabolic dysfunction marked by hyperglycaemia as a result of absence of secretion of insulin by pancreatic β-cells, inefficient insulin usage by the body, or both [1-3]. Globally, diabetes has been reported to affect estimates of 415 million in 2015, 425 million in 2017 and 463 million persons in 2019. This number is expected to surge to 578 and 700 million in 2030 and 2045, respectively with prevalence in low and middle-income countries than in high-income countries [3,4]. Diabetes mellitus can only be managed, not cured and majority of the available synthetic antidiabetics are accompanied with serious side effects hence, the need for investigation of new drugs from natural sources is highly essential [5-7].

Traditional medicine often offers polyherbal therapy with individual component plants managing different symptoms and exerting its effect on different organs. This concept is similar to the theory of poly-pharmacy [8]. The use of herbs as combination therapy in the management of several disease conditions such as cancer, acquired immunodeficiency syndrome (AIDS), malaria, pulmonary, tuberculosis, diabetes mellitus, etc has resulted in many outcomes. These include optimum activity at lower dose than individual plants, additional pharmacological effect exerted by the individual components of the extract, synergistic effect of the different components in the extract and ease of administration resulting in a better adherence to herbal drug use to achieve enhanced therapeutic effects [9]. Although, herb-herb combinations have been used in traditional medicine practice for several years, scientific understanding of various herb-herb interactions or proof of clinical benefits was slow and weak resulting in the need for further research in herbal combination therapy [8]. Poly-herbal formulations in the management of diabetes mellitus have been variously reported [10-15].

Senecio biafrae Oliv. & Hiern (Asteraceae), known as 'worowo' in Yoruba (Nigeria) and 'Gnanvule' (Coted'Ivoire) [16] is used ethnomedically as galactagogue [17] and in the management of diabetes in combination with the seeds of *Aframomum melegueta* and *Carica papaya* [18]. Its antihyperglycaemic, hypolipidemic and antioxidant activities have been reported [19,20,21]. *Xylopia aethiopica* (Dunal) A. Rich (Annonaceae) is used in Nigeria to manage diabetes, hypertension, hyperlipidaemia and obesity [22]. It has been reported for its antihyperglycaemic [23], antiproliferative [24] and hypolipidemic and antioxidant potentials [25]. Different parts of *Carica papaya* L. (Caricaceae) are used in the management of diabetes, hypertension and stroke [22]. It has been reported for its antihyperglycemic, anti-inflammatory and antioxidant activities [26], larvicidal [27], antisickling [28] activities. *Spondias mombin* L. (Anacardiaceae) is used in Nigeria and African countries in the management of diabetes [18,22,29,30].

These plants have been individually studied for their antidiabetic activities in our research laboratories and the findings have been reported [21,23,26,31]. This work was therefore designed to investigate the antidiabetic activities of the combined extract of the active plant parts in equal ratio and its safety in order to discover the possible advantage(s) of the combined plant extract over the individual plants.

2.0. MATERIALS AND METHODS

2.1. Chemical Equipment and Instrumentation

Rotary evaporator (RE301/601/801 model, Yamato Scientific America, Inc., U.S.A), chiller (Churchill, Instrument Co. Ltd, U.K), vacuum pump (MB 338618 model, Edwards High Vacuum Int., England), oven (Hearson & Co. Ltd, London), Mettler electronic weighing balance (AB 54 model, Mettler Toledo, U.S.A), Ultra-violet (UV) lamp (254 and 366 nm) (Grant Instrument, U.K), Oral cannula, ACCU-CHEK Glucometer (model GB 11558973, Roche, Germany) with ACCU-CHECK test strips (Roche, Germany), UV spectrophotometer, Dutrao (Model SM 600, Shang Yhai Yong Chuang Medical Instrument Co. Ltd) spetrophotometric microplate reader, Automated haematology analyzer, Centrifuge, Semi-automated biochemistry analyzer.

2.2. Plant Materials and Extraction

Senecio biafrae leaf, *Carica papaya* seed and *Spondias mombin* stem bark were collected on Obafemi Awolowo University campus while *Xylopia aethiopica* fruit was purchased from Oja Oba market, Modakeke, Osun State. They were authenticated and their voucher specimens with herbarium specimen numbers, FPI 2324, 2323, 2325 and 2326, respectively were deposited at the Faculty of Pharmacy Herbarium. The various morphological plant parts were separately air-dried, powdered and mixed together in ratio 1:1:1:1. A total of 2600 g of plant material (containing 650 g each of the individual plant part) was extracted by maceration in methanol with mechanical agitation. The marc was re-extracted three times and concentrated *in-vacuo* to obtain a yield of 20.2 % w/w.

2.3. Animals

Healthy Wistar rats (120-180 g) of both sexes were bred under standard conditions (temperature 27-30°C, relative humidity 65 %) in the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria. They were given regular pellets diet (Bendel Feeds, Nigeria) and water *ad libitum*. Ethical clearance number IPH/OAU/12/1758 was issued by the Health Research Ethics Committee, Institute of Public Health, Obafemi Awolowo University on the use of animals for this work.

2.4. Acute Toxicity Test

The acute toxicity test was carried out according to the modified OECD Test Guideline 423 Annex 3 model [32]. Two groups of 8 animals each were administered distilled water and single oral administration of 5000 mg/kg extract, respectively. They were observed for signs of gross toxicity, behavioural changes and mortality, one hour after administration and daily for 14 days [32].

2.5. Subacute Toxicity Test

Sub acute toxicity test was carried out following the modified OECD Test Guideline 407 [33]. The extract was solubilised in 1 % Tween 80 in distilled water and administered to groups of 8 rats daily for 28 days at graded doses of 250, 500, and 1000 mg/kg. The blood glucose levels in rats were monitored on days 7, 14, 21 and 28. The animals were anaesthetized using chloroform and blood sample (5 mL) collected by cardiac puncture after the 28^{th} day [33].

2.6. Haematological Analysis

About 50 µL of blood was aspirated into the automated haematology analyzer for haematological analysis [34].

2.7. Biochemical Assays

In the assay of transaminases (Aspartate Transaminase (AST), Alanine Transaminase (ALT)), serum (100 µL) was added to 1000 µL of working solution of SGOT R1 and R2 (4:1) and the mixture was aspirated into the analyser to obtain the absorbance for the AST. While 100 µL of serum was added to 1000 µL of working solution of SGPT R1 and R2 (4:1) and the mixture was aspirated into the analyser to obtain the absorbance for the ALT Serum (20 µL) was added to 1000 µL of working solution containing one tablet of ALP substrate and 10 mL of ALP diluents and the solutions were aspirated into the analyser to obtain the absorbance of the blank and test.

1000 µL of working solution containing equal volume of creatinine picrate and diluents was placed inside three test tubes for blank, standard and test for the Creatinine test. Serum sample (50 µL) was added into the tube for test while creatinine standard (50 µL) was added into the tube for standard. The solutions were aspirated into the analyser to obtain the absorbance of the blank, standard and test. Serum (10 µL) and urea standard (10 µL) were added to working solution containing urea R1 and R2 (4:1) placed inside two test tubes for the standard and test. The solutions were aspirated into the analyser to obtain the absorbance of the standard and test. Serum (10 µL), cholesterol standard (10 µL) were added into the tubes for test and sample containing 1000 µL working solution of cholesterol reagent. The mixtures were incubated at 37^0C for 10 minutes after which they were aspirated into the analyser and the absorbance for the blank, standard and test were obtained.

2.8. Antidiabetic Studies

2.8.1. In-vitro α-amylase inhibitory activity of the extract

The assay mixture consists of 1.0 mL of 0.020 M solution of sodium phosphate buffer (pH 6.90 with 0.0060M NaCl), 1 mL alpha amylase solution (from *Aspergillus oryzae*) and 0.4 mL extract at different concentration (0.05, 0.1, 0.25, 0.5, 1.0 mg/mL). Pre-incubation was done at 37 ºC for 10 minutes, followed by the addition of 1 mL of 1 % solution of boiled potato starch into the tubes. The reaction mixture was kept at 37°C for another 15 minutes before being stopped with 1 mL of 3,5-dinitrosalicylicacid (DNSA) (containing 1.00 g of 3,5-dinitrosalicylicacid, 20.0 mL of 2.0 M NaOH and 30.0 g of sodium potassium tartarate in 100 mL distilled water). After 5 minutes in a boiling water bath, the tubes were cooled to room temperature. The mixture was diluted with 5 mL distilled water, and the absorbance was measured with a spectrophotometer at 540 nm. With the exception of the extract, a control indicating 100 % enzyme activity was carried out in a similar manner. The synthesis of reducing sugar occurs when 3,5-dinitrosalicylicacid is reduced to 3-amino-5-nitrosaliylicacid. Acarbose was used as the positive control. The following was used to calculate the alpha amylase inhibition and represented as a % of inhibition:

$$\text{Inhibition (\%)}=[([(A_{c+})-(Ac-)]-[A_s-A_b])/\{(A_{c+})-(A_{c-})\}]*100$$

Where:

A_{c+}= absorbance of 100 % enzyme activity,

A_{c-}= 0 % enzyme activity (only solvent without enzyme),

A_s= test sample (with enzyme) and

A_b= blank (a test sample without enzyme) [35,36].

2.8.2. *In-vitro* α-glucosidase inhibitory activity of the extract

The α-glucosidase inhibitory assay was carried out with a Dutrao spectrophotometric microplate reader (Model SM 600, Shangyhai Yong chuang Medical Instrument Co. Ltd). A total of 60.0 µL of reaction mixture was added to each well, consisting of 20.0 µL of 100.0 mM phosphate buffer (pH 6.80), 20.0 µL of 2.50 mM (pNPG) 4-Nitrophenyl-D-glucopyranoside, and 20.0 µL

methanol dissolved sample, afterwards 20.0 µL of 10.0 mM phosphate buffer (pH 6.80) containing 0.20 U/mL alpha glucosidase to the mixture in the wells. The reaction was incubated at 37°C and terminated with 80.0 µL of 0.20 mM sodium carbonate 15 min later. The absorbance was estimated using a microplate reader at 405.00 nm. The above procedure was repeated using acarbose as the positive control [37].

2.8.3. Antihyperglycaemic effect of extract on glucose induced hyperglycaemic rats

Groups of 5 rats each fasted for 18 hours that were given 10 g/kg of glucose (p.o.) were used for the experiment. After 0.5 hour (time point 0), rats having blood glucose levels ≥7.0 mmol/L (126 mg/dL) were considered hyperglycaemic and given (*p.o.*) vehicle (Tween 80 (1 %) in distilled water) (negative control) extract (25, 50, 100 and 200 mg/kg) separately and 5.0 mg/kg glibenclamide (positive control). At 0.00, 0.50, 1.00, 2.00, and 4.00 hours, blood drop from each rat's caudal vein was placed on to a glucometer strip inserted into the glucometer. The percentage decrease in blood glucose level at these time points was calculated and compared to the negative and positive controls [38-41].

2.8.4. Antihyperglycaemic effect of extract on streptozotocin-induced diabetic rats

Streptozotocin (65 mg/kg) in freshly prepared buffer solution (0.1M, pH 4.5) was intraperitoneally administered to overnight fasted rats to induce diabetes. After 72 hours of induction, the rats' blood glucose levels were measured, and they were then left for another 5 days. Rats with Fasting Blood Sugar ≥ 11.0 mMol/L were considered diabetic and divided into four groups of five rats: negative control, administered with 1 % Tween 80 in distilled water; test groups, 50 and 100 mg/kg and positive control, glibenclamide (5 mg/kg). On days 1, 4, 7, 10, and 14, blood glucose levels were measured and compared to the control group [5].

2.9. In vitro Antioxidant Studies

2.9.1. 1,1-dipheny1-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH or radical scavenging properties of the extract was determined using the stable radical 1,1-dipheny1-2-picrylhydrazyl (DPPH) according to [26,39,42]. The absorbance was then measured at 517 nm against a DPPH control containing l ml of methanol in place of the extract. The scavenging activity was then calculated using the formula below:

$$\text{Percentage scavenging activity}=\frac{absorbance\ control-absorbance\ sample}{absordance\ control}\ \text{x}\ 100$$

The $\mathbf{IC_{50}}$ was then obtained from a linear regression plot of percentage inhibition against concentration of extract.

2.9.2. Evaluation of Total Antioxidant Capacity (TAC)

The total antioxidant assay was carried out based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (v) complex at acidic pH [43].

2.9.3. Ferric Reducing Antioxidant Power (FRAP)

The FRAP assay uses antioxidant as reductants in redox linked colorimetric method [44].

2.9.4. Hydroxyl Radical Scavenging Activity (HRSA)

The ability of the extracts to scavenge the hydroxyl radical generated by the Fenton reaction was measured according to the modified method of Ferrer-Sueta and Radi [45].

2.9.5. Determination of Total Flavonoid Content

The estimation of the Total Flavonoid Content of the extract was based on the Aluminium chloride colorimetric method according to the method of [46] and as described by [47]. The result was expressed as mg rutin equivalent (RE)/g of the extract. Analysis was done in triplicates.

2.9.6. Total Phenolic Content (TPC)

The total phenol content of the extracts was determined using Folin-Ciocalteu's method of [48] Singleton as described by Gulcin [49]. The principle is based on reduction of phosphomolybdic-phosphotungistic acid (Folin reagents) to a blue-coloured complex in an alkaline solution which occurs in the presence of phenolic compounds.

2.10. Statistical analysis.

Data were expressed as the mean ± SEM for the number (n) of animals in the group. Analysis of variance (ANOVA) then Student Newman Keul's test was used to obtain the source of significant differences for all determination. $P < 0.050$ was considered as statistically significant.

3.0. RESULTS AND DISCUSSION

3.1. Acute Effect of Extract in Normal Rats

The result of the acute toxicity study of the methanol extract showed no observable changes in behaviour of the rats with respect to breathing, cutaneous effect, sensory and nervous system responses or gastrointestinal effect. No mortality or gross toxicity was also observed. Thus, the median lethal dose, LD_{50} was estimated to be greater than 5000 mg/kg, suggesting that the extract is safe and possesses low risk of toxicity. It also indicated that the doses of 25-1000 mg/kg used in the study were experimentally safe. Extracts of *S. biafrae, X. aethiopica, C. papaya* and *S. mombin* had been reported to be safe between 2000 and 5000 mg/kg [21,50-52].

3.2. Sub acute Effect of the Extract on Blood Glucose Level in Normal Rats

The negative control normoglycaemic rats that were given distilled water did not show any appreciable decrease in the level of blood glucose of the rats throughout the period of the study confirming that the water did not contain any bioactive constituents. The extract of the plant combination at the tested doses of 250, 500 and 1000 mg/kg and for all the days of the experiment had comparable ($p>0.05$) effect on the blood glucose level of the rats to the negative control (Table 1). This indicated safety of the extract and that it cannot precipitate hypoglycaemic coma when administered to non diabetic individuals. Similar effect has been reported for *Eugenia uniflora, Olax subscorpioidea, Entandrophragma cylindricun, Triclisia subcordata* [40,41,53,54].

3.3. Effect of Extract on Haematological Parameters

Investigation of haematological components of humans/animals, such as red blood cells, white blood cells or leucocytes, mean corpuscular volume, mean corpuscular haemoglobin and mean

corpuscular haemoglobin concentration is valuable in monitoring toxicity as well as the health status of animals [55]. Administration of 250, 500, 1000 mg/kg of the extract to rats for 28 days in this study showed a significant increase in RBC level which suggested positive effect on the haemopoietic system of the test rats indicating possible anti-anaemic effect of the extract (Table 2). However, it gave no significant difference in other evaluated haematological parameters when compared to the control. This indicated that the extract had no adverse effect on blood components and further established its lack of toxicity. Extracts of *Mangifera indica* stem bark and *Telfaria occidentalis* had been reported to show similar effects on the haematological components in rats [56,57].

3.4. Effect of Extract on Biochemical Parameters

The results of the biochemical analysis of the blood samples after treatment for 28 days showed comparable activity ($p > 0.050$) of the extract on aspartate transaminase (AST), alanine transaminase (ALT), creatinine and urea level to distilled water (negative control) (Table 3). This result indicated the extract does not have any toxic effect on the heart, liver and kidney of the rats. High AST and ALT levels have been associated with liver diseases or hepatotoxicity [58,59]. The extract however consistently elicited a reduction in cholesterol level at all the tested doses compared to negative control showing possible antihyperlipidemic effect of the extract. Hypolipidaemic activity has been reported for *Senecio biafrae, Carica papaya and Xylopia aethiopica* [19,20,25] which confirmed the findings of this study.

3.5. *In vitro* α-amylase Inhibitory Effect of the Extract

Pancreatic α-amylase and α-glucosidase play a critical role in carbohydrate digestion and effective inhibitors of these enzymes such as acarbose, miglitol and voglibose are being used in the management of diabetes [60-62]. However, their side effects necessitated a search for new α-amylase and α-glucosidase inhibitors of natural origin without side effects and to provide more candidates of drug choices. In the α-amylase inhibitory assay in this study, the extract showed a significant concentration dependent antihyperglycaemic effect from 0.05-1.0 mg/mL similar to acarbose. The extract with IC_{50} of 0.06 mg/mL that was lower than 0.1 mg/mL of the positive control indicated better α-amylase inhibitory antihyperglycaemic potential of the extract (Table 4).

3.6. *In vitro* α-glucosidase Inhibitory Effect of Extract

Similar to the α-amylase inhibitory ~~antihyperglycaemic~~ result (Table 4), the extract exhibited a concentration dependent antihyperglycaemic activity that was comparable to the positive control (Table 5). The IC_{50} values of 0.17 ± 0.02 and 0.19 ± 0.01 for the extract and acarbose, respectively indicated the extract ~~like~~ similar to acarbose effectively inhibited the breaking down of complex carbohydrates to glucose thereby preventing glucose assimilation from the intestine into the bloodstream [26,63]. This confirmed the extrapancreatic activity in addition to the insulin stimulation effect that had been reported for *Senecio biafrae* [21] and the α-amylase and α-glucosidase inhibitory activities reported for *Carica papaya* [26].

3.7. *In Vivo* anti-hyperglycaemic Activity of the Extract

The results of glucose-loaded rat model or Oral Glucose Tolerance Test (OGTT) model with glibenclamide and other insulin-stimulating drugs as positive controls have been reported to

mimic type 2 diabetes state in humans and reportedly used in anti-hyperglycaemic assays of medicinal plants [6,7,64,65]. The glucose loaded hyperglycaemic rats that received distilled water (negative control) gave a significant ($p < 0.050$) time-dependent decrease in blood glucose level till the fourth hour due to homeostatic regulatory process in normal animals (Fig.1) [6,26,66]. Generally, the extract at the tested doses of 25, 50, 100 and 200 mg/kg elicited a time dependent antihyperglycaemic effect till the fourth hour similar to glibenclamide (5 mg/kg) which suggested that the extract may have early extrapancreatic and late insulin stimulating mechanism of action of glibenclamide [41,53,54,67]. The activity of the extract was dose dependent with maximum effect at 100 mg/kg that was significantly better ($p < 0.050$) than glibenclamide (5 mg/kg) at all time points which indicated an additional extrapancreatic effect of the extract at this dose (Fig.1). High blood glucose level reduction of 19, 40 and 43 % observed between 0.5-2 h demonstrated by 100 mg/kg of the extract which indicated better extrapancreatic effect was justified by the high α-amylase and α-glucosidase inhibitory activities exhibited by the extract (Tables 4 and 5). In the previous work done on the individual plants on this combination [21,23,26], highest antihyperglycaemic effect was obtained at 400 mg/kg. This suggested that the antihyperglycaemic activity of the component plants was highly enhanced in the combination and showed optimum activity at lower dose of 100 mg/kg indicating synergism (Fig.1).

3.8. Effect of the Extract on Streptozotocin-induced Diabetic Rats

The most active dose in the glucose-induced antihyperglycaemic experiment and its lower dose, 100 and 50 mg/kg, respectively (Fig. 1) were used in this experiment. The non treated diabetic rats consistently maintained an hyperglycaemic state throughout the study period showing that the diabetes induced by the administered streptozotocin was permanent (Fig. 2). Glibenclamide (5 mg/kg), the positive control drug gave a time dependent antidiabetic activity of 13, 56, 75, 79 % on days 4, 7, 10 and 14, respectively that was due to insulin stimulating action of the drug on the remaining pancreatic β-cells of the diabetic rats (Fig. 2). The extract of the plant combination at 50 mg/kg, with 31, 85 and 85 % antidiabetic effect on days 4, 7 and 10, respectively were significantly higher than the 14, 56 and 75 % activity of glibenclamide on the same days and 71 and 72 % of 100 mg/kg on days 7 and 10. This indicated a better activity of the extract at 50 mg/kg (Fig. 2). This observation further confirmed that the antidiabetic effect of the combination plant extract was significantly better at lower doses than the individual plants which was one of the advantages of poly-herbal therapy [9,18,21,23,31].

3.9.Antioxidant Activity of the Extract

The antioxidant activity of the extract was determined using multiple assays (DPPH, FRAP, TAC, HRSA, TPC and TFC) in order to determine the possible contribution of antioxidant effect of the extract to its antidiabetic activity [21]. In the DPPH assay (Table 6), the extract gave an IC_{50} value of 0.341 mg/ml which showed a better radical scavenging effect than that reported for *S. biafrae* leaf and *C. papaya* seeds alone [21,26]. The combined plant extract with values 17.4 mgAAEq/g and IC_{50} of 0.368 mg/ml for FRAP and HRSA assays indicated better activity than that reported for *S. biafrae* and *C. papaya* seeds alone [21,26]. The antioxidant activity of the extract was significantly higher in the TAC assay compared to that reported for *C. papaya, X. aethiopica, S.biafrae and S. mombin* alone. The extract also showed high values for TFC and TPC (Table 6), indicating that it was rich in phenolics and flavonoids which was confirmed for *S. biafrae, C. papaya* and *S. mombin* [21,26,68].

Table 1: Effect of extract on blood glucose level in sub acute toxicity study in normal rats

Extract/Drug (mg/kg)	Day 1	Day 7	Day14	Day 21	Day 28
DW	100	95.36±3.37[a]	94.26±4.45[a]	99.34±3.00[a]	100.26±2.81[a]
SXCS (250)	100	102.31±7.00[a] (-7.29%)	94.59±4.83[a] (-0.35%)	107.05±9.00[a] (-7.76%)	89.49±5.24[a] (10.74%)
SXCS (500)	100	94.66±4.18[a] (0.73%)	91.92±5.54[a] (2.48%)	102.50±3.16[a] (-3.18%)	85.57±3.61[a] (14.65%)
SXCS (1000)	100	94.81±2.71[a] (0.58%)	102.48±1.75[a] (-8.72%)	104.88±6.18[a] (-5.58%)	96.58±4.45[a] (3.67%)

Data show the mean ± SEM blood glucose levels at the different time points (Tt) expressed as percentages of level at day 1, percentage reductions in the bgls relative to negative control for each time point, N = 8. Values with similar superscript are comparable (p>0.05). One-way analysis of variance followed by the Student-Newman-Keuls' post-hoc test). **DW**: Distilled Water; **SXCS** (250, 500, 1000): *S. biafrae*, *X. aethiopica*, *C. papaya*, *S. mombin* extract.

Table 2: Effect of extract on haematological parameters in sub acute toxicity study in normal rats

Blood Parameters	D W	SXCS(250 mg/kg)	SXCS(500 mg/kg)	SXCS(1000 mg/kg)
WBC (10^3/μL)	15.49±0.82[a]	18.34±1.27[a]	15.34±1.22[a]	18.34±2.40[a]
RBC (10^6/μL)	6.60±0.22[a]	7.30±0.22[a,b]	7.41±0.30[a,b]	7.90±0.43[b]
HGB (g/dL)	12.16±0.33[a]	12.86±0.31[a]	13.20±0.19[a]	12.49±0.84[a]
HCT (%)	46.66±1.91[a]	48.79±1.80[a]	46.17±1.84[a]	50.68±2.62[a]
MCV(fl)	70.66±1.12[b]	66.73±1.26[a]	62.39±0.79[a]	64.33±1.92[a]
MCH(pg)	18.51±0.49[a]	17.64±0.41[a]	18.01±0.85[a]	16.08±1.30[a]
MCHC(%)	26.24±0.79[a]	26.34±0.54[a]	28.89±1.26[a]	24.89±1.81[a]
Platelet(10^3/μL)	444.25±77.09[a]	498.29±87.89[a]	454.29±79.78[a]	644.50±94.01[a]

Data show the mean ± SEM haematological parameters at the different doses, n=8. Results having separate superscripts within row are significantly different (p < 0.050), while those that are alike are comparable (p > 0.050): One-way variance analysis (ANOVA) followed by Student-Newman-Keul's test. **DW:** Distilled Water; **SXCS** (250, 500, 1000): *S. biafrae*, *X.*

aethiopica, *C. papaya*, *S. mombin* extract. **WBC**: White Blood Corpuscles, **RBC**: Red Blood Corpuscles, **HGB**: Haemoglobin, **HCT**: Haematocrit, **MCV**: Mean Corpuscular Volume, **MCH**: Mean Corpuscular Haemoglobin, **MCHC**: Mean Corpuscular Haemoglobin Concentration.

Table 3: Effect of extract on biochemical parameters in sub acute toxicity study in normal rats

Biochemical Parameters	D W	SXCS (250 mg/kg)	SXCS (500 mg/kg)	SXCS (1000 mg/kg)
AST(μ/L)	351.95±14.08[a]	339.00±29.92[a]	287.15±17.26[a]	367.85±20.62[a]
ALT(μ/L)	65.87±3.26[a]	57.17±5.33[a]	53.98±3.36[a]	60.82±4.10[a]
CREA(mg/dL)	6.77±0.10[a]	6.47±0.19[a]	5.65±0.41[a]	6.20±0.42[a]
CHOL(mg/dL)	256.55±18.89[b]	196.55±17.12[a]	199.20±7.32[a]	201.20±5.73[a]
ALP(IU/L)	149.87±24.84[b]	138.41±12.56[a,b]	81.42±12.42[a]	119.23±15.18[a,b]
UREA(mg/dL)	23.81±0.71[a]	23.56±0.37[a]	22.49±0.05[a]	22.98±0.12[a]

Data show the mean ± SEM biochemical parameters at different doses, n=8. Results having separate superscripts within row are significantly different ($p < 0.050$), while those that are alike are comparable ($p > 0.05$). **DW**: Distilled Water; **SXCS** (250, 500, 1000): Extract of *S. biafrae*, *X. aethiopica*, *C. papaya*, *S. mombin*. **AST**: Aspartate Transaminase, **ALT**: Alanine Transaminase **CREA**: Creatinine, **CHOL:** Cholesterol, **ALP:** Alkaline phosphatase

Table 4: *In vitro* *α*-amylase inhibitory effect of extract

Concentration of extract and Acarbose (mg/ml)	Average % Inhibition		IC_{50} (mg/kg)	
	Extract	Acarbose	Extract	Acarbose
1	85.63[a]	83.09[a]	0.06±0.02[a]	0.10±0.01[a]
0.5	77.59[a]	80.2[a]		
0.25	68.73[b]	63.03 [a]		
0.1	64.75[b]	55.31[a]		
0.05	35.45[a]	46.60[b]		

Data show the mean ± SEM *α*-amylase inhibitory effect of extract at different concentrations, n=3. Values with different superscripts within rows are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test); Results with separate

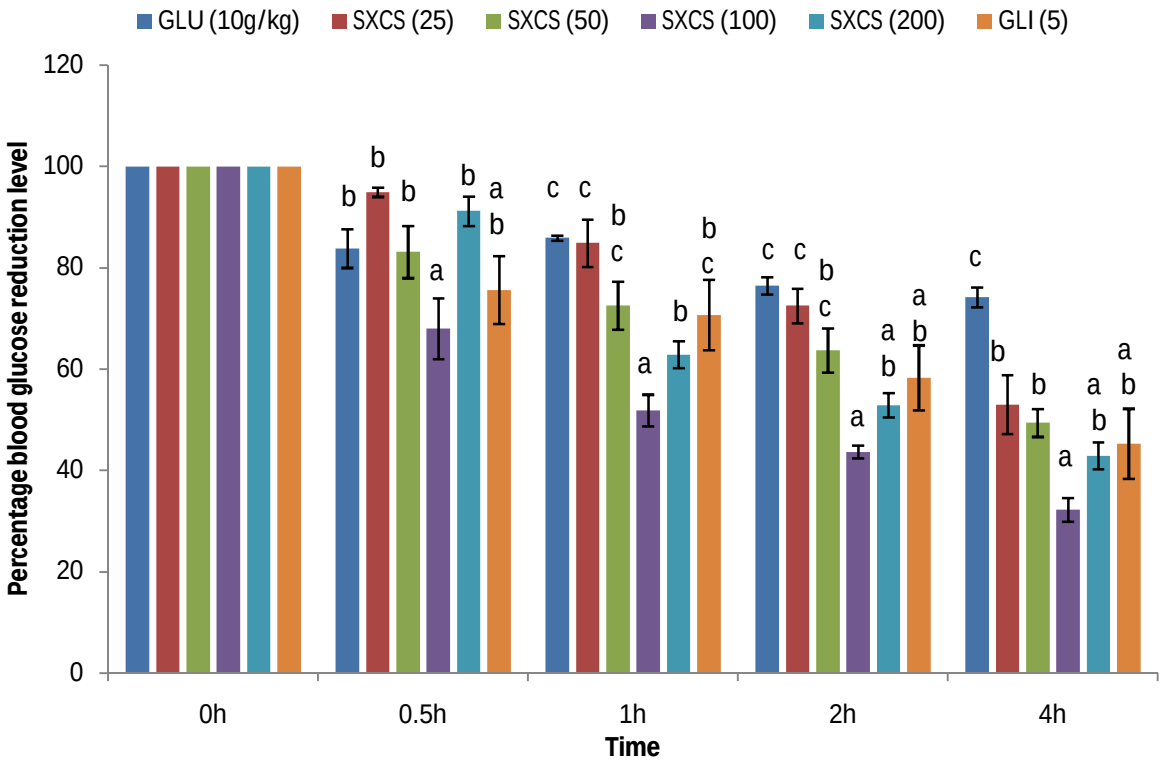
superscripts within rows are significantly different ($p < 0.050$), while those that are alike are comparable ($p > 0.05$).

Table 5: *In vitro* α-glucosidase inhibitory effect of extract

Concentration of extract and Acarbose (mg/mL)	Average % Inhibition		IC_{50} (mg/mL)	
	Extract	Acarbose	Extract	Acarbose
0.5	68.21[a]	89.52[b]	0.17±0.02[a]	0.19±0.01[a]
0.25	58.24[a]	59.43[a]		
0.125	48.64[b]	40.69[a]		
0.0625	38.86[b]	31.80[a]		
0.03125	11.21[a]	12.40[a]		

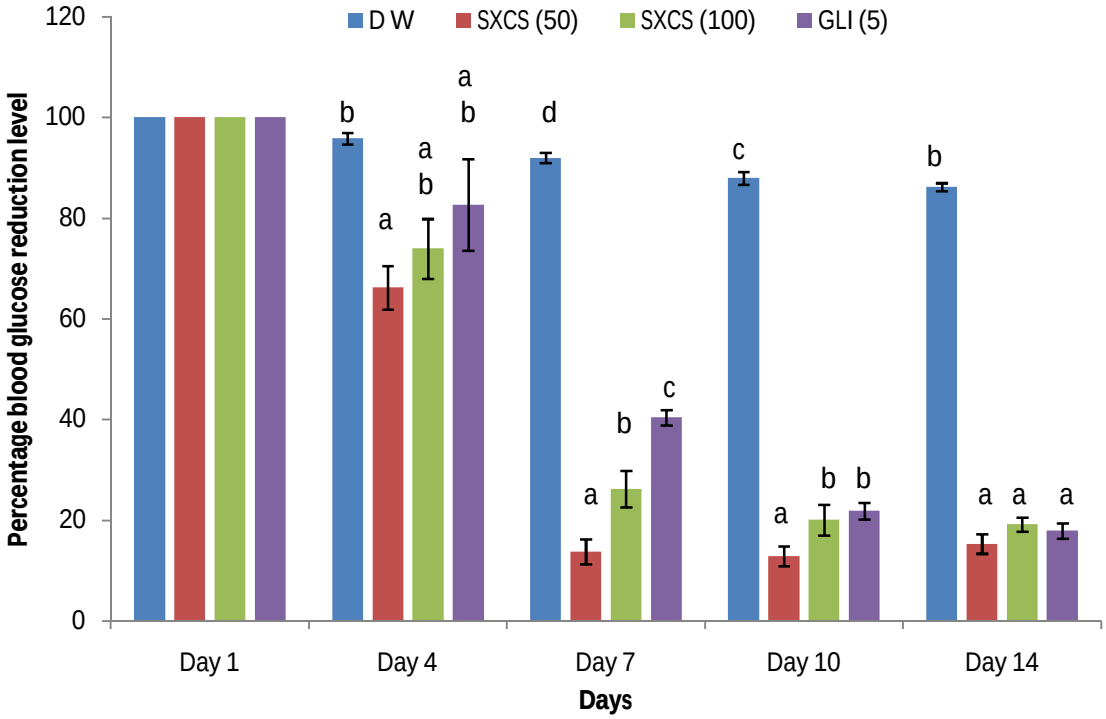
Data show the mean ± SEM α-glucosidase inhibitory effect of extract at different concentrations, n=3. Values with different superscripts within rows are significantly different ($p < 0.05$, one-way analysis of variance followed by the Student–Newman–Keuls' test); Results with separate superscripts within rows are significantly different ($p < 0.050$), while those that are alike are comparable ($p > 0.05$).

Figure 1. Dose related antihyperglycaemic effect of extract in rats



Data shows the mean ± SEM levels of blood glucose at the various period of time represented in percentage, n=5. Results having separate superscripts are significantly different ($p < 0.050$), while those that are similar are comparable ($p > 0.050$): One-way variance analysis (ANOVA) then Student-Newman-Keul's test. **GLU** (10 g/kg): Glucose 10 g/kg; **SXCS (25-200):** *S. biafrae*, *X. aethiopica*, *C. papaya*, *S. mombin* extract GLI: Glibenclamide (5.0 mg/kg).

Figure 2. Effect of the extract on streptozotocin-induced diabetic rats



Data show the Mean ± SEM level of blood glucose at the various period of time expressed as percentage, n=5. Results having separate superscripts are significantly different ($p < 0.050$), while those that are alike are comparable ($p > 0.050$): One-way variance analysis (ANOVA) then Student-Newman-Keul's test. **DW**: Distilled Water; **SXCS (50, 100):** *S. biafrae*, *X. aethiopica*, *C. papaya*, *S. mombin* extract , **GLI (5):** Glibenclamide (5.0 mg/kg).

Table 6: Antioxidant activity of the extract

Extract/Drug	IC_{50} (mg/mL)	(mg GAEq/g)	(mg REq/g)	(mgAAEq/g)		IC_{50} (mg/mL)
	DPPH	**TPC**	**TFC**	**TAC**	**FRAP**	**HRSA**
SXCS	0.341	54.7 ± 0.04	69.5 ± 1.4	30.1 ± 1.7	17.41 ± 0.7	0.368
Ascorbic Acid	0.113					0.168

Data show the mean ± SEM (n=3). IC_{50} concentration needed to give 50% activity; mgAAEq/g: mg Ascorbic acid equivalent per g; mgGAEq/g: mg Gallic acid equivalent per g; mg REq/g: mg Rutin equivalent per g. DPPH: (1,1-dipheny1-2-picrylhydrazyl) radical scavenging activity assay; FRAP: Ferric Reducing Antioxidant Power; TAC: Total Antioxidant Capacity; HRSA: Hydroxyl Radical Scavenging Activity; TPC: Total Phenolic Content; TFC: Total Flavonoid Content, **SXCS**: *S. biafrae*, *X. aethiopica*, *C. papaya*, *S. mombin* extract.

The overall blood glucose level reduction elicited by the combination plant extract in this study could be adduced to both its α-amylase and α-glucosidase inhibitory (extra pancreatic) activities (Tables 4 and 5), insulin stimulation (Fig. 1 and 2) as well as free radical scavenging activity (Table 6). *Senecio biafrae*, *Carica papaya*, *Xylopia aethiopica* and *Spondias mombin* had been reported to exert their anti-hyperglycaemic effects through extra pancreatic and insulin stimulating mechanisms of action with additional anti-oxidant effects [18,21,23,25,31,69]. Furthemore, the anti-hyperglycaemic and antioxidant activities elicited by the combined plant extract were significantly higher than those given by each of the component plants.

3. CONCLUSION

The results of this study confirmed that the combination of *Senecio biafrae* leaf, *Xylopia aethiopica* fruit, *Carica papaya* seed and *Spondias mombin* stem bark mixed together in equal ratio had significant antidiabetic activity at lower doses than the individual plants without toxic effects on glucose levels, haematological and biochemical components of animal blood samples. It also confirmed that the combined plant extract will be more beneficial in the management of diabetes mellitus than the component plants and could therefore be used as an antidiabetic recipe.

ACKNOWLEDGEMENTS

The authors are grateful to Mr. Ogunlowo Ifeoluwa, Department of Pharmacognosy, O.A.U., Ile-Ife, Nigeria for his assistance in the collection of the plant materials. The study was not funded by any company or funding agency but by individual efforts of the authors.

CONFLICT OF INTEREST

The authors of this study hereby declare that there was no conflict of interests in the course of this work.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. As M.Sc and B.Pharm research students, author OIB and OO did the research work, while author MDA was their supervisor and author KFA carried out the biochemical and antioxidant work. OIB and MDA wrote the manuscript, read by all authors and approved for publication, while author MDA processed for publication.

CONSENT

This is not applicable to this study.

ETHICAL APPROVAL

Ethical clearance number IPH/OAU/12/1758 was issued by the Health Research Ethics Committee, Institute of Public Health, Obafemi Awolowo University on the use of animals for this work.

This is not applicable to this study.

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