The Journal of Phytopharmacology 2019; 8(3): 129-134
Online at: www.phytopharmajournal.com

Research Article

ISSN 2320-480X
JPHYTO 2019; 8(3): 129-134
May- June
Received: 15-04-2019
Accepted: 20-06-2019

DOI: 10.31254/phyto.2019.8308

Ighodaro OM
Biochemistry Laboratory, Lead City University, Nigeria

Asejeje FO
Biochemistry Laboratory, Lead City University, Nigeria

Adeosun AM
Biochemistry Laboratory, Lead City University, Nigeria

Ujomu T
Department of Pharmacology &Therapeutics, University of Ibadan, Nigeria

Bakre SR
Biochemistry Laboratory, Lead City University, Nigeria

Correspondence:
Ighodaro OM
Biochemistry Laboratory, Lead City University, Nigeria
Email: macigho[at]gmail.com

Anti-diabetic potential and gas chromatography mass spectroscopy (GC-MS) profile of a formulated polyherbal drug (FPD)

Ighodaro OM, Asejeje FO, Adeosun AM, Ujomu T, Bakre SR

ABSTRACT

Background: Conventional treatments for diabetes mellitus apparently lack the desired therapeutic interventions and are relatively expensive for a number of individuals suffering from the diseases. Hence, several formulated polyherbal drugs are currently utilized as treatment options for diabetes. **Objective**: In view of this, the present study investigated the acclaimed antidiabetic potential of a formulated polyherbal drug (FPD) popularly sold in a major city (Ibadan) in Nigeria as *Merry Herbal Tea*. FPD was screened for its active constituents and antidiabetic efficacy using GC-MS technique and alloxan-induced diabetic rats respectively. **Methods:** The diabetic rats were randomized into a diabetic control group and three diabetic groups separately treated with FPD (5mL/kg), FPD (10mL/kg) and Metformin (12mg/kg) BW (p.o) twice daily, for a period of five days. Blood glucose (FBGL) was monitored at different time intervals using acucheck glucometer and pancreas was harvested from each animal and processed for histological examinations. **Results:** Although FPD at the studied doses lowered blood glucose levels in the experimental rats but unlike metformin, its hypoglycemic effect was not significant ($P<0.05$) when compared to the initial diabetic glucose level of the animals. This observation was substantiated by the pancreatic histological architectures of the FPD and metformin treated animals relative to their untreated diabetic counterparts. Conversely, FDP significantly lowered the number of diabetes-induced mortality (28.6 %) relative to the untreated diabetic group (60.1%). Contained in FDP among other bioactive compounds is Lup-20(29)-en-3-ol, acetate which has been associated with diabetic functions in previous studies. **Conclusion:** The blood glucose and mortality lowering effects, as well as the presence of Lup-20(29)-en-3-ol, acetate demonstrated by FPD within the evaluation period tend to suggest a possible antidiabetic relevance for the formulated polyherbal drug. However, we recommend that the antidiabetic effects of FDP be assessed for a longer period and more assays related to diabetes be conducted before a definite conclusion is made on its antidiabetic relevance.

Keywords: Diabetes, Alloxan, Polyherbal, Wistar rats, GC-MS.

INTRODUCTION

Diabetes mellitus (DM) remains a major global health challenge and its spread across different age cadres is deeply worrisome. For various reasons, the number of people with DM is incredibly increasing [1] and more than ever before diabetic patients are in dire need of any treatment option proffered as a solution to their life-threatening health conditions. In light of this, various kinds of polyherbal formulations with antidiabetic claims are popularly sold at different locations in many cities within the country (Nigeria). Interestingly, in some cases individuals have provided empirical and clinical evidences to support the efficacy of such formulations. On the contrary, a couple of these formulations do not possess the desired and acclaimed therapeutic effects. Such polyherbal formulations would rather worsen the health conditions of the users (patients) and endanger their lives.

In the city of Ibadan, the capital city of Oyo state, Nigeria, a formulated polyherbal drug packaged in Lipton tea-like bag is commonly sold as *Merry Herbal Tea* to people suffering from diabetes mellitus and its associated complications. With the uncertainty associated with these alternative therapies, it is expedient that they are screened or investigated scientifically in order to help the public make informed decisions as regards their choice of antidiabetic drugs. In this regards, alloxan monohydrate remains one of the commonly used diabetogenic models for assessing the antidiabetic or hypoglycemic capacity of test compounds [2]. Notably, alloxan is far less expensive and more readily available compared to other diabetogenic agents such as streptozotocin. In the present study, alloxan-induced diabetes was employed in assessing the antidiabetic efficacy of the said formulated polyherbal drug (FPD) using Wistar rat as

a model organism, and the chemical constituents of FPD were determined using gas chromatography – mass spectrometry (GC-MS) procedure.

MATERIAL AND METHODS

Collection and Management of Animals

Forty-two adult male rats (mean body weight of 200 ± 7.2 g) were purchased from the Animal Breeding Unit of the Department of Anatomy, University of Ibadan. They were managed according to the according to the procedure outlined by the National Academy of Science published by the National Institute of Health [3], approved by the Animal Research Ethics Committee of Faculty of Basic Medical & Applied Sciences, Lead City University, Ibadan Nigeria, for the use of animals in research. The animals were handled humanely, kept in a wooden cage, placed in a well ventilated and hygienic rat house under suitable conditions of temperature and humidity. They were acclimatized for two weeks prior to commencement of study. The animals were subjected to natural photoperiod of 12 hours light and 12 hours dark cycle, and provided rats pallets (Top feeds) and water *ad libitum.* All animal experiments were carried out without anesthesia during the study.

Collection and preparation of drugs

Metformin (an antidiabetic drug) was obtained from Gilead pharmaceutical store, Challenge, Ibadan and used as a reference drug. 2.4 mg of the drug was dissolved in 1mL of distilled water to obtain a dose of 12mg/kg of body weight. The formulated polyherbal drug (FPD), packaged in Lipton tea-like bag is a popularly sold herbal drug in Ibadan metropolis. It is a combination of two or more plant materials and is acclaimed to be a highly effective treatment for diabetes mellitus. The herbal drug was extracted with hot water according to the manufacturer`s instruction and the doses adopted were based on recommended volume per 70kg body weight. For instance, 350 mL of the extract is taken as a single dose by an adult (average weight of 70kg). Hence, 5mL/kg and 10 mL/kg BW were used as normal dose and high dose in this study. Alloxan monohydrate solution was freshly prepared by dissolving 36mg of alloxan in 1 mL of saline to obtain a dose of 180mg/kg of body weight (BW). Normal saline was prepared by dissolving 0.9g of sodium chloride (NaCl) in 100 mL of distilled water.

Induction of Diabetes mellitus, animal grouping and treatment

Diabetes mellitus was experimentally induced in thirty-five animals by single intraperitoneal injection of alloxan monohydrate (180 mg/kg of BW). The remaining seven animals were used as normal control group (group **i**). Rats were fasted overnight prior to induction of diabetes. 48 hours after alloxan administration, following an overnight fasting, diabetes was confirmed in the animals. Rats with blood glucose level greater than 200 mg/dL were considered diabetic [4] and randomized into four (4) groups (**ii, iii, iv and v**) containing seven animals each (n=7). The animals were immediately placed on 5% glucose solution in order to prevent hypoglycemic shock usually associated with alloxan intake. Groups **ii** animals served as diabetic control, groups **iii** and **iv** were respectively treated with 5 and 10 mL/kg BW of the investigated formulated polyherbal drug and group **v** animals were treated with metformin (12mg/kg BW). All treatments were done orally, twice daily at 8 h interval for a period of five days.

Monitoring of Blood Glucose level

Rat blood glucose was obtained through the tail and estimated using accucheck active glucometer with disposable test strips. Prior to collection of blood, the tail was cleaned with surgical spirit and then nibbed with a pair of sharp scissors. A test strip was fully inserted into the glucometer before applying a drop of blood to fully cover a test area inside the grey target. The test area of the strip works in such a way that a drop of blood on its surface causes color changes which is proportional to the concentration of glucose in the blood sample. After collection of blood, the animal was protected against infection and further bleeding by rubbing the nibbed side of the tail with surgical spirit -soaked cotton wool. Blood glucose level was monitored at different time intervals. The first phase was done at1 h interval for six hours and the second phase was done on the first, third and 5th day post confirmation of diabetes in the animals. After the last glucose reading, the animals were sacrificed by cervical dislocation and the pancreas was harvested from each animal and processed with 10% (v/v) formalin solution for histopathological examination.

Gas chromatography-mass spectroscopy (GC-MS) analysis of FPD

The packed formulated polyherbal drug (FPD) was soaked in 100 mL of methanol for 48 h, the filtrate was obtained and concentrated on water bath. The concentrated extract was then subjected to GC-MS analysis [5].

Statistical Analysis

The statistical significance of difference between groups were analyzed with One-Way Analysis of Variance (ANOVA) followed by independent-sample t test, using a statistical software tool, Prism Graphpad (version 6.4) at 95% confidence level ($P < 0.05$).

RESULTS

Effect of FPD treatment on blood glucose level in Diabetic Wistar rats

Tables 1 and 2 show the effects of formulated polyherbal drug (FPD) treatment on blood fasting blood glucose level in alloxan-induced diabetic rats within the first six hours and five days respectively. Formulated polyherbal drug (FPD) at the studied doses (5 mL/kg BW and 10mL/kg BW), unlike metformin caused non-significant reduction in blood glucose in the experimental rats. It however significantly lowered the rate of diabetes-induced mortality when compared to the untreated diabetic group of animals (diabetic control group). 60.1% mortality was recorded in the group of untreated diabetic animals (group ii), 33.3% in diabetic animal treated with normal dose (5 mL/kg BW) of FPD and 28.6 % in diabetic animals groups respectively treated with high dose (10 mL/kg BW) of FPD and Metformin (12mg/kg BW).

Table 1: Blood glucose level of rats within 6h post treatment of Diabetic Wistar rats with FPD

Group	Initial blood glucose level (mg/dL)	Blood glucose level (mg/dL) at different post treatment duration (h)					
		1	2	3	4	5	6
I	89 ± 5.3	91 ± 2.3	94 ± 5.1	87 ± 3.8	84 ± 6.1	76± 4.2	85 ± 3.9
Ii	467 ± 23.6	472 ± 45.3	503 ± 32.7	514 ± 62.8	497 ± 28.9	465 ± 56.8	472 ± 48.7
Iii	>600	>600	>600	>600	>600	>600	>600
Iv	444 ± 25.3	435 ± 38.9	450 ± 15.6	534 ± 49.3	473± 28.7	423 ± 14.1	433 ± 19.2
V	530 ± 15.7	510 ± 12.4	540 ± 21.1	497 ± 33.8	530 ± 15.7	470 ± 32.9	540 ± 17.6

Values are expressed as mean ± standard deviation (SD) of 4 rats. Group i = control (normoglycemic animals treated with saline), Group ii = Diabetic control (untreated diabetic animals), Group iii = Diabetes + FPD_{ND} (Diabetic animals treated with 5mL/kg BW of FPD), Group iv = Diabetes + FPD_{HD} (Diabetic animals treated with 10mL/kg BW of FPD), Group v = Diabetes + Metformin (Diabetic animals treated with 12mg/kg BW of Metformin), FPD = formulated polyherbal drug, ND = Normal dose, HD = High dose

Table 2: Blood glucose level of rats within 3 days post treatment of diabetic Wistar rats with FPD

Group	Initial glucose level (mg/dL)	Blood glucose level (mg/dL) at different post treatment duration (h)		
		Day 1	Day 3	Day 5
I	89 ± 5.3	81 ± 3.4	79 ± 6.3	92 ± 7.8
ii	467 ± 23.6	502 ± 35.3	498 ± 42.8	466 ± 24.7
iii	>600	567 ± 35.1	559 ± 19.3	478 ± 29.1
iv	444 ± 25.3	435 ± 26.9	407 ± 18.1	383 ± 29.2
V	530 ± 15.7	410 ± 22.3	340 ± 11.8	267 ± 13.8

Values are expressed as mean ± standard deviation (SD) of 4 rats. Group i = control (normoglycemic animals treated with saline), Group ii = Diabetic control (untreated diabetic animals), Group iii = Diabetes + FPD_{ND} (Diabetic animals treated with 5mL/kg BW of FPD), Group iv = Diabetes + FPD_{HD} (Diabetic animals treated with 10mL/kg BW of FPD), Group v = Diabetes + Metformin (Diabetic animals treated with 12mg/kg BW of Metformin), FPD = formulated polyherbal drug, ND = Normal dose, HD = High dose

Effects of FPD treatment on pancreas histological architecture of Diabetic Wistar rats

The photomicrographs of thin sections (5µm) of pancreas of normoglycemic rats as well as those of alloxan-induced diabetic rats treated with FPD and metformin are shown in Figs1A to 1E. The pancreas of the control animals showed normal histological architecture (Fig. 1A) whereas the pancreas of diabetic animals (treated and untreated) showed different degrees of histological aberrations (Fig. 1A to 1E), with most severe damage noted in the untreated diabetic group (Fig. 1B), followed by FDP-treated and metformin-treated groups respectively.

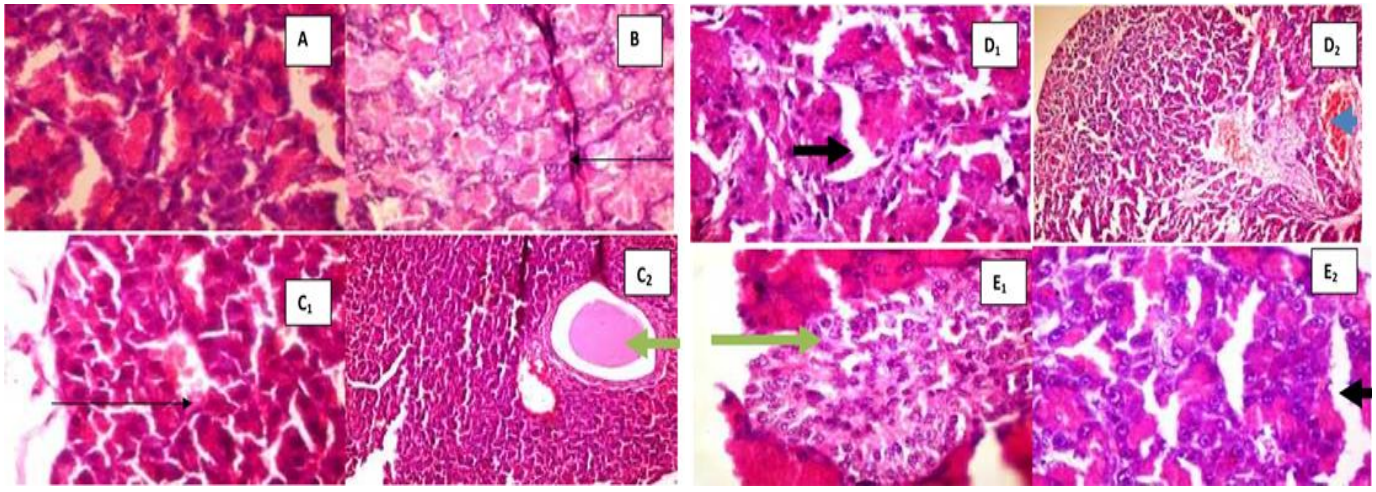

Figure 1: Photomicrograaphs of the pncreas of control and experimental rats (A): Pancreas of normoglycemic rats (Stained with H & E, X400) showing normal histological architecture (B): Pancreas of diabetic rats left untreated (Stained with H & E, X400), plate shows absence of islet beta cells, and the exocrine acini contain pale stainning zymogen granules (slender arrows) (C_1) & (C_2): Pancreas of diabetic rats treated with normal dose of FPD (Stained with H & E, X400), plates show absence of islet beta cells and the exocrine acini shows picnotic nuclei with basophilic cytoplasm (slender arrows). focal area of eosinophilic secretion in the intralobular ducts (green arrows), (D_1) & (D_2): Pancreas of diabetic rats treated with high dose of FPD (Stained with H & E, X400). Plates show extensive area of architectural anarchy with picnotic nuclei (black arrows) and disseminated congestion (blue arrows). (E_1) & (E_2): Pancreas of diabetic rats treated with Metformin (Stained with H & E, X400). Plates show islets with preponderance of atypical cells (the nuclei sappear abnormal and chromatin enlarged)(green arrows), the cells of the exocrine acini appear enlarged and vesicular (black arrows).

Biologically active compounds detected in FPD using gc-ms technique

The GC-MS chromatogram is represented by Figure 2. The result shows the presence of ten (10) active compounds in the analyzed formulated polyherbal drug (FPD). The main compounds contained in FPD in terms of abundance and known biological activity are Benzene, 1,1'-(1,3-propanediyl) bis, Benzene, 1,1'-(1-methyl-1,3-propanediyl) bis, squalene, Octadecanoic acid, Lup-20(29)-en-3-ol, acetate, 2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one, Methyl-Z,Z-3,13-octadecadienol cis-Vaccenic acid,Oleic Acid (9-Octadecenoic acid/ trans-13-Octadecenoic acid) and Hexadecanoic acid, ethyl ester (Table 3). The chromatogram and structures of some of the compounds are shown in Figs 3 and 4.

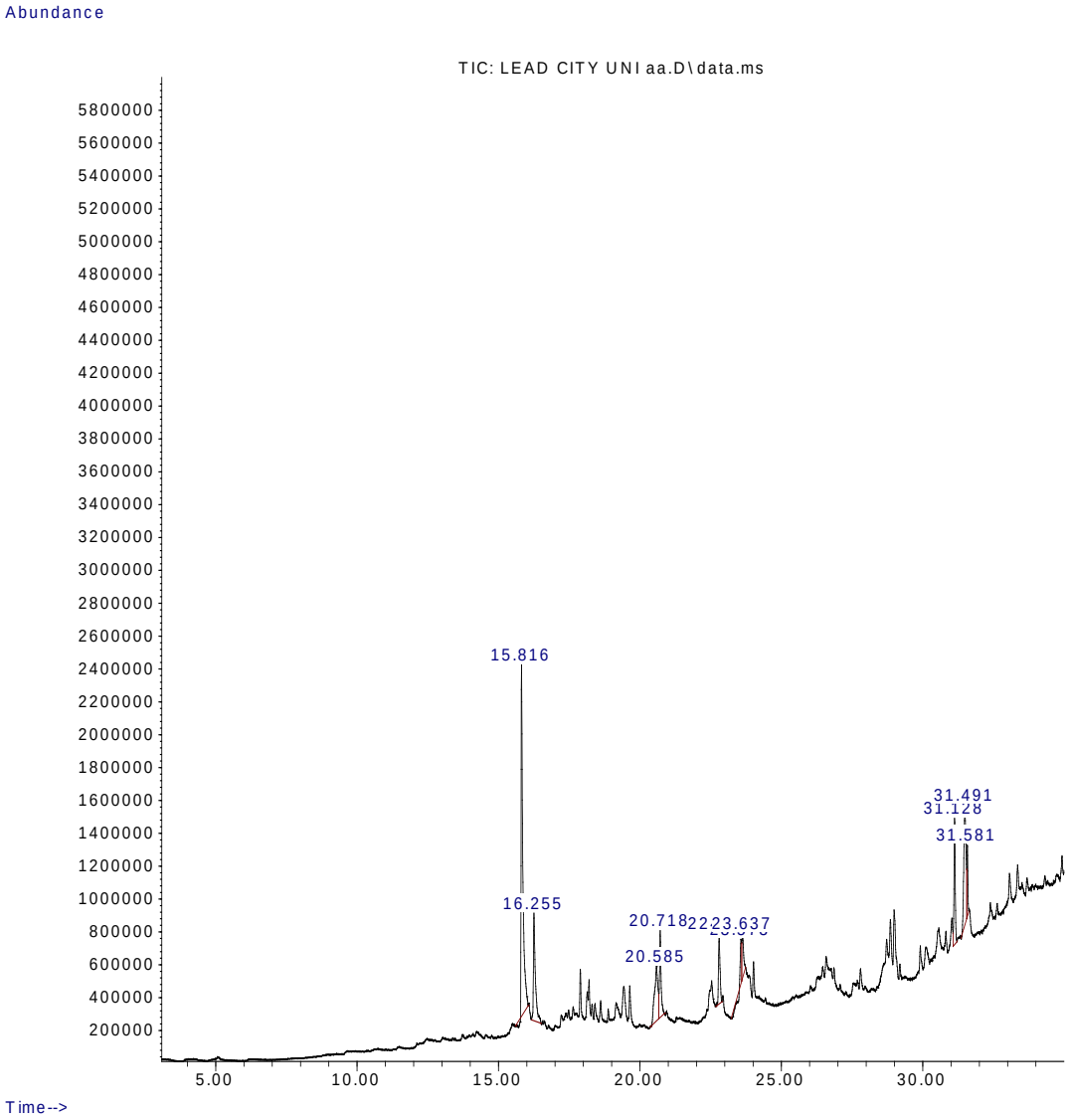

Figure 2: GC-MS Chromatogram of a formulated polyherbal drug commonly sold as an antidiabetic drug in Ibadan metropolis

Table 3: Compounds detected in a formulated Polyherbal drug using GC-MS Technique

S/N	Compound	Retention time (min:s)	Percentage abundance (%)
1	Benzene, 1,1'-(1,3-propanediyl)bis	15:82	32.70
2	Benzene, 1,1'-(1-methyl-1,3-propanediyl) bis-	16:25	11.24
3	Octadecanoic acid	20:59	9.99
4	Hexadecanoic acid, ethyl ester	20:72	7.49
5	Tridecanedial/Phytol/ Neophytadiene	22:80	5.91
6	Methyl-Z,Z-3,13-octadecadienol cis-Vaccenic acid	23.57	1.72
7	Oleic Acid	23:63	3.97
8	Squalene	31:13	8.57
9	Lup-20(29)-en-3-ol, acetate/ 2,2,6-Trimethyl-1-(2-methyl-cyclobut-2-enyl)-hepta-4,6-dien-3-one	31:49	14.09
10	Eicosane/ Heptacosane, 1-chloro	31:58	3.12

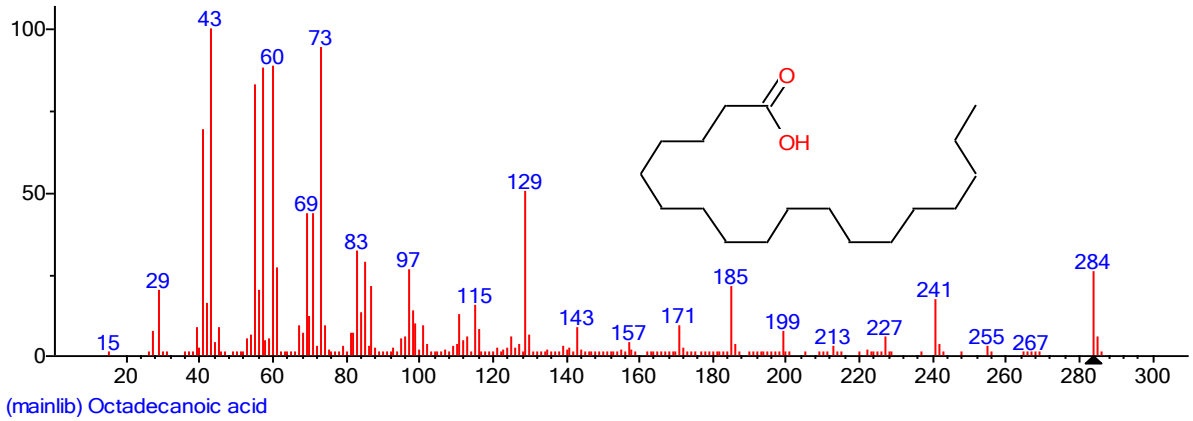

Figure 3: GC-MS Chromatogram showing the structure of octadenoic acid

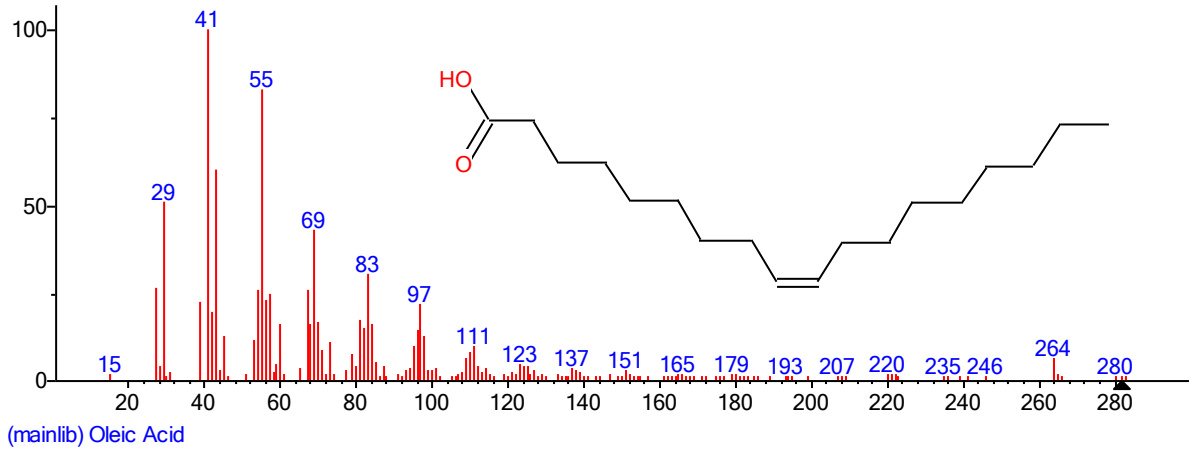

Figure 5: GC-MS Chromatogram showing the structure of Oleic acid

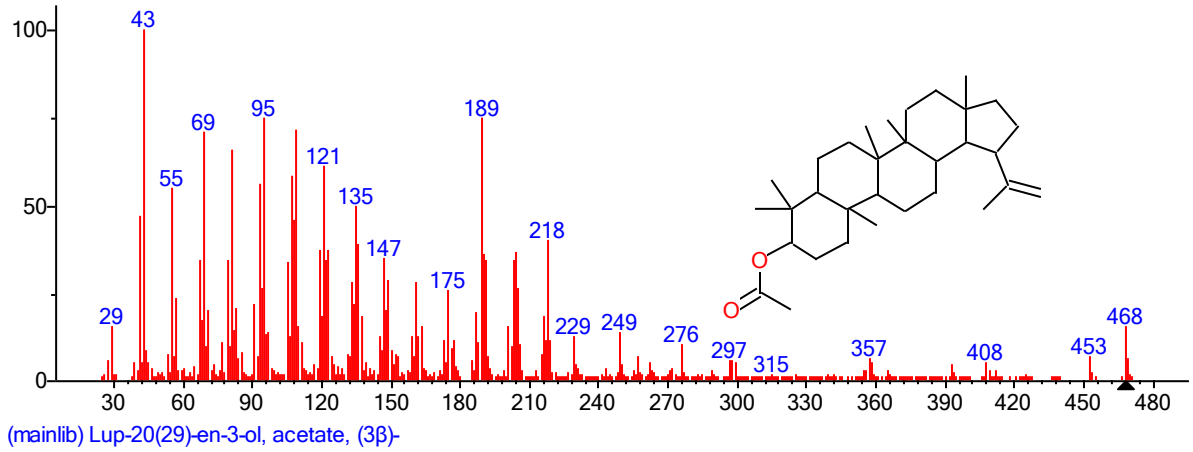

Figure 6: GC-MS Chromatogram showing the structure of Lup-20(29)-en-3-ol,acetate,(3b)

DISCUSSION

Increased reliance on formulated polyherbal drugs by a number of people suffering from diabetes is obviously a cause for concern. Arguably, some plant extracts have been found to be highly effective against the ailments or diseases for which they are prescribed or recommended. In support of this, previous studies [6-8] have demonstrated that some herbal extracts have potent hypoglycemic activity when administered orally in normal and diabetic rats.

Conversely and probably for monetary purpose or other selfish reasons, the `pharmaceutical market` is continuously flooded with some polyherbal drugs which do not possess the medicinal relevance ascribed to them. This wrong practice does not only mislead the uninformed patients who are in dire need of therapeutic intervention for their failing health but also worsen their health conditions and endanger their lives.

In view of the above, the present study was undertaken to ascertain the therapeutic efficacy of a formulated polyherbal drug (FPD) popularly sold in a major city in Nigeria vis-a-vis its impact on diabetes and the diabetics. The data obtained when analyzed showed that oral administration of the investigated formulated polyherbal drug (FPD) at the adopted doses did not elicit any notable hypoglycemic activity in

the model organism (Wistar rat). This observation is at variance with some previous studies in which investigated polyherbal formulations were found to exhibit hypoglycemic effects in either alloxan or streptozotocin-induced diabetic rats [9-11].

However, FPD was noted to have significantly lowered the number of diabetes-induced mortality when compared to the untreated diabetic group of animals (diabetic control group). This attribute is suggestive of plausible antidiabetic effect for the investigated polyherbal drug if administered for a period longer than the five days employed in this study. This notion is supported by the presence of Lup-20(29)-en-3-ol acetate which has been previously associated with diabetic functions [4, 12] in FPD. Moreover, Metformin used as a reference drug in this study is an established antidiabetic agent. Hence, similarities in the results obtained for both FPD-treatment and Metformin-treatment of the diabetic animals further buttress a possible antidiabetic relevance for FPD.

CONCLUSION

Although the hypoglycemic effects of the investigated formulated polyherbal drug (FPD) was not significant, the presence of Lup-20(29)-en-3-ol, acetate in the compound as well as its ability to significantly reduced diabetes-induced mortality tend to suggest a possible antidiabetic function for the formulated polyherbal drug. We therefore recommend that the antidiabetic effects of FDP be assessed for a longer period than the duration employed in the present study before a definite conclusion can be made on its antidiabetic relevance.

Conflict of Interest

The authors declare that there is no conflict of interest as regards this article

REFERENCES

1. Farag YM, Gaballa MR. Diabesity: an overview of a rising epidemic. Nephrology Dialysis Transplantation, 2010; 26(1):28-35.
2. Ighodaro OM, Adeosun AM, Akinloye OA. Alloxan-induced diabetes, a common model for evaluating the glycemic-control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina*, 2018.
3. NIH Publication Number 04–4158 February 2010 Available at http://www.win.niddk.nih.gov
4. Ighodaro OM, Akinloye OA. Anti-diabetic potential of S apium ellipticum (Hochst) Pax leaf extract in Streptozotocin (STZ)-induced diabetic Wistar rats. BMC complementary and alternative medicine, 2017; 17(1):525.
5. Karasek FW, Clement RE. *Basic gas chromatography-mass spectrometry: principles and techniques*. Elsevier, 2012.
6. Asante DB, Effah-Yeboah E, Barnes P, Abban HA, Ameyaw EO, Boampong JN, *et al*. Antidiabetic effect of young and old ethanolic leaf extracts of Vernonia amygdalina: A comparative study. Journal of diabetes research, 2016.
7. Li Y, Wang C, Huai Q, Guo F, Liu L, Feng R, *et al*. Effects of tea or tea extract on metabolic profiles in patients with type 2 diabetes mellitus: a meta-analysis of ten randomized controlled trials. Diabetes/metabolism research and reviews, 2016; 32(1):2-10.
8. Gholamnezhad Z, Havakhah S, Boskabady MH. Preclinical and clinical effects of Nigella sativa and its constituent, thymoquinone: A review. Journal of Ethnopharmacology. 2016; 190:372-386.
9. Karigar Asif A, Shariff WR. Antidiabetic effect of polyherbal formulation in alloxaninduced diabetes mellitus. Journal of Pharmacy Research. 2009; 2(11):1757-1759.
10. Sabbani V, Alluri R, Satla S. Evaluation of hypoglycemic and anti–diabetic activity of poly-herbal formulation in normal and streptozotocin induced diabetic rats. The FASEB Journal. 2013; 27(1 Supplement):1169-4.
11. Chaudhuri A, Sharma S. Evaluation of Antidiabetic Activity of Polyherbal Formulation in Streptozotocin-Induced Diabetic Rats. UK Journal of Pharmaceutical and Biosciences. 2016; 4(5):01-06.
12. Lakshmi V, Mahdi AA, Ahmad MK, Agarwal SK, Srivastava AK. Antidiabetic activity of lupeol and lupeol esters in streptozotocin-induced diabetic rats. Bangladesh Pharmaceutical Journal. 2015; 17(2):138-146.

HOW TO CITE THIS ARTICLE

Ighodaro OM, Asejeje FO, Adeosun AM, Ujomu T, Bakre SR. Anti-diabetic potential and gas chromatography mass spectroscopy (GC-MS) profile of a formulated polyherbal drug (FPD). J Phytopharmacol 2019; 8(3):129-134.