Journal of Diabetes and Metabolism

Ezekwe et al., J Diabetes Metab 2017, 8:5
DOI: 10.4172/2155-6156.1000740

Research Article OMICS International

GC-MS Analysis, Hypoglycemic Activity of Aqueous Root Extract of *Carica papaya* and Its Effects on Blood Lipid Profile and Hepatorenal Tissues Biomarkers of Diabetic Rats

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Received date: May 03, 2017; **Accepted date:** May 10, 2017; **Published date:** May 17, 2017

Abstract

The likely phytocomponents, present in aqueous root extract (AqRE) of *Carica papaya*, responsible for hypoglycemic activity and their capacity to reverse altered blood lipid profile and compromised hepatorenal functions in alloxan-induced diabetic rats were investigated. The identification of main phytocomponents of AqRE of *C. papaya* was carried out using GC-MS system. The capacity of AqRE of *C. papaya* to reverse altered blood lipid profile and compromised hepatorenal functions were investigated using standard diagnostic techniques. The relative abundance of the 7 major phytocomponents present in AqRE of *C. papaya* was within the range of 3.49-35.63%. AqRE of *C. papaya* lowered elevated fasting blood glucose concentration of the diabetic rat group by 30.95%. The diabetic rat group treated with AqRE of *C. papaya* gave absolute values of TC-(HDL-C)]/(HDL-C=1.15 units, TC/HDL-C=2.15 units and LDL-C/HDL-C=1.56 units. Additionally, the ratio of serum ALT to AST activities of the diabetic rat group treated with AqRE of *C. papaya* gave absolute value of 2.27 units. Serum total bilirubin, creatinine and urea concentrations of the diabetic rat group treated with AqRE of *C. papaya* were significantly lower ($p<0.05$) than that of diabetic rat group. GC-MS chromatogram of AqRE of *C. papaya* revealed the presence of phytochemicals of therapeutic potentials. AqRE of *C. papaya* ameliorated hyperglycemia and dyslipidemia in the diabetic rats. Finally, AqRE of *C. papaya* exhibited greater capacity to ameliorate obstructive liver dysfunction than hepatic tissue injuries in the diabetic rats.

Keywords: *Carica papaya*; Diabetic rats; GC-MS analysis; Hepatorenal; Lipid profile

Introduction

Diabetes mellitus (DM) is a typical metabolic disease usually characterized by hyperglycemia, engendered by defects in insulin secretion (Insulin-Dependent Diabetes Mellitus; IDDM or Type I DM) and peripheral tissue resistance to insulin (Non-Insulin-Dependent Diabetes Mellitus; NIDDM or Type II DM) or both [1,2]. Gestational DM is associated with raised level of oxidative stress in pregnancy [3]. Chronic hyperglycemia in concert with dyslipidemia elicits hyperoxidative stress, which in turn, have been implicated in the pathogenesis of micro- and macrovascular complications such as retinopathy, nephropathy, neuropathy, myocardial infarction and atherosclerosis in DM state [4-6]. Furthermore, DM pathophysiology manifest in form of tissue/organ dysfunctions with multidimensional alterations in cellular metabolism [7,8]. Epidemiological survey showed that DM, among other dilapidating pathologic conditions, is the leading cause of morbidity and mortality of all age groups worldwide [9]. In the year 2000, the World Health Organization reported that 2.8% of global population was afflicted with DM and epidemiological projections showed that the number is expected to rise to 366 million (4.4% of global population) by 2030 [10]. These statistics obviously underscore the enormous public health concerns of DM pathology, which calls for the development of new anti-diabetic remedies and improvement in already established anti-diabetic therapies for attainment of global health and wellness. Studies have shown that patients suffering from chronic diseases such as DM are turning away from the use synthetic drugs to herbal remedies as alternative therapeutic strategy [11,12].

Carica papaya L., Caricaceae (papaya) is a perennial herbaceous tropical plant with prominent leaves (20-60 cm long) [13] and the semi-woody, latex-producing and usually single stem may attain a height up to 10 meters. The papaya root is predominantly a non-axial fibrous system composed of one or two tap roots of 0.5-1.0 meter long. *Carica papaya* (papaya) is widely distributed throughout the tropics and subtropics of the world, where the prevalence of DM is high [13]. The Carica latex contains endo-peptidases I and II [14], alkaloids such as carpaine, pseudocarpaine, dehydrocarpaine I and II as well as kaempferol, quercetin and p-coumaric acid in the leaves [15,16]. There are summarized reports on the therapeutic properties of *C. papaya* extracts [17]. For instance, leaf extract of *C. papaya* is an effective anti-sickling agent [18-20], exhibits anti-inflammatory activity [21,22], possesses larvicidal activity against *Aedes aegypti* [23] and is effective in the treatment of dengue [24].

Extracts from plant materials are often used in form of fractions and isolated preparations in research protocols, which to a large extent are composed of mixtures of numerous bioactive principles [25,26]. Isolated and characterized bioactive principles from plants are of tremendous resources for new drug discoveries and clinical research such as in specialized fields of pharmacognosy and medicine. Additionally, novel bioactive principles from plant materials are necessary tools for the development of synthetic and semi-synthetic

compounds for the advancement of modern therapeutic strategies [27].

The capacity of *C. papaya* extracts to lower elevated plasma glucose concentration in normal and DM patients have been severally reported elsewhere [13,28-30]. However, these studies were not exhaustive [30] in that the likely bioactive principles responsible for hypoglycemic activity were not identified but often reported in generalized and qualitative terms. Gas Chromatography-Mass Spectrometry (GC-MS) offers an efficient protocol for identification and characterization bioactive principles [26,30-33]. Using GC-MS analytical methods, in concert with standard diagnostic techniques, the present study was designed to identify the likely bioactive principles, present in aqueous root extract (AqRE) of *C. papaya*, responsible for hypoglycemic activity in alloxan-induced diabetic rats. Additionally, the capacity of AqRE of *C. papaya* to reverse altered blood lipid profile as well as organ/tissue pathology such as compromised hepatorenal functions in the experimental rats were also carried out.

Materials and Methods

Collection of plant specimen

Roots of matured and fruit bearing *C. papaya* were harvested during the wet season, on the 6th of July, 2015 from Imo State University, Owerri botanical garden (Latitude 5°30.2237'N; Longitude 7°2.6277'E), which lies on the rainforest belt of Nigeria. The harvested roots were identified and authenticated by Dr. F.N. Mbagwu at the Herbarium (Sample Voucher Number: IMSUH 196), Department of Plant Science and Biotechnology, Imo State University, Owerri, Nigeria. Thereafter, the roots were washed under continuous current of tap water for 5 min to remove soil matter and air-dried at ambient room temperature (T=25 ± 5°C) for 24 h.

Preparation and extraction

A 100-g part of chopped fresh roots were weighed using a triple beam balance (OHAU 750-50; OHAUS Triple Beam Balance, Model TJ611, Burlington, NC, USA) and dried to constant weight in an oven (WTC BINDER; 7200 Tuttlingen, Germany) at 50°C for 10-12 h as previously described [34]. The dried samples were ground into powder using the Thomas-Willey milling machine (ASTM D-3182; India) and sieved on a wire mesh screen (3 × 3 mm^2). Finally, the powdered sample was stored at refrigerated temperature of 4°C in air-tight plastic bottles with screw caps pending extraction.

Preparation of AqRE of *C. papaya* was according to the methods previously described by Ibegbulem and Chikezie, [2]. Twenty-five grams (25 g) of the ground sample was suspended in 250 mL of distilled water in stoppered flasks and allowed to stand for 24 h. The suspension was filtered with Whatman No 24 filter paper. The filtrate was concentrated in a rotary evaporator (Büch Rotavapor R-200) for 12 h at 50°C under reduced pressure and dried in vacuum desiccator. The yield was calculated to be 5.61% w/w. Portion of the extract was finally suspended in phosphate buffered saline (PBS) solution (extract vehicle), osmotically equivalent to 100 g/L NaCI {NaCI (90.00 g), Na_2HPO_4.2H2O (17.10 g) and NaH_2PO_4.2H2O (2.43 g)/L} and subsequently administered to experimental rats. According to the methods of Nguyen and Kimaru, [35], another portion of the extract was suspended in ethyl acetate and subjected to GC-MS analysis.

GC-MS system

The identification of main bioactive principles of AqRE of *C. papaya* was carried out using GC-MS detection system as previously described [36] but with minor modification, whereby portion of the extract was analyzed directly by headspace sampling. GC-MS analysis was performed using an Agilent 7890A GC system equipped with 5975C VL MSD (Agilent Technologies, CA, USA). The capillary column used in this study was DB-5MS (30 m × 0.25 mm, film thickness of 0.25 µm; J&W Scientific, CA, USA). The temperature program was set as follows initial temperature 50°C held for 1 min, 5°C per min to 100°C, 9°C per min to 200°C held for 7.89 min, and the total run time was 30 min. The flow rate of helium as a carrier gas was 0.811851 mL/min. The MS system was operated in electron ionization (EI) mode with selected ion monitoring (SIM). The ion source temperature and quadruple temperature were set at 230°C and 150°C, respectively. Identification of phytocomponents was performed by comparison of their retention times and mass with those of authentic standards spectra using computer searches in NIST08.L and Wiley7n.l libraries.

Experimental animals/ethics

The male albino (Wistar) rats (8-10 weeks old; average weight of 118 ± 2.1 g) were obtained from the Animal House of Imo State University, Owerri, Nigeria. The rats were housed in well-ventilated metal cages and maintained at room temperature of 28 ± 2°C, 30 - 55% of relative humidity on a 12-h light/12-h dark cycle, with access to water and pelletized standard guinea feed (PSGF) (United Africa Company Nigeria Plc., Jos, Nigeria) ad libitum for 2 weeks acclimatization period. The present study was approved by the Ethical Committee on the use of animals for research, Department of Biochemistry, Imo State University, Owerri, Nigeria (Ethics Approval Number: ODVC/REN/998/15). Handling of the rats was in accordance with the standard principles of laboratory animal care of the United States National Institutes of Health (NIH, 1978).

Induction of diabetes mellitus and study design

Induction of DM and study design was according to previous report [37]. DM was induced in the experimental rats by single intra-peritoneal (i.p.) injection of 90 mg/kg body weight (bw) of alloxan monohydrate (Sigma-Aldrich, St. Louis, MO, USA) suspended in PBS solution (pH=7.4). The animals with fasting plasma glucose concentration (FPGC)>110 mg/dL for 5 consecutive days were considered for the study. A total of 24 male Wistar rats were allotted into 4 groups of 6 rats each. The animals were deprived of food and water for an additional 16 hours prior to the commencement of treatment as previously described [2]. The animal groups were designated on the basis of treatments received at regular intervals of 7 days for 21 days as previously described [38].

- Group 1: Normal rats received PSGF + water ad libitum+PBS (1.0 mL/kg bw; i.p.).
- Group 2: DM rats received PSGF + water ad libitum+PBS (1.0 mL/kg bw; i.p.).
- Group 3: DM rats received PSGF + water ad libitum+AqRE of *C. papaya* (500 mg/kg bw in PBS; i.p.).
- Group 4: DM rats received PSGF + water ad libitum +glibenclamide (5.0 mg/kg bw in PBS; i.p.).

Blood volumes of 0.05 mL were drawn from the tails of the experimental rat groups at regular intervals of 7 days, prior to each

consecutive treatment, for 21 days and measured for fasting plasma glucose concentration (FPGC). Furthermore, at the end of the treatment period, blood volume of 3.0 mL were drawn from 12-hour post-fasted experimental rat groups and measured for serum lipid profile (SLP) and hepatorenal tissues biomarkers, namely, plasma aspartate aminotransferase (AST) activity, alanine aminotransferase (ALT) activity, alkaline phosphatase (ALP) activity, total bilirubin concentration, urea concentration and creatinine concentration.

Preparation of blood samples

Plasma was separated from whole blood in Na_2EDTA anti-coagulant test tube by centrifugation at 1500x g at 4°C for 10 min [39]. Serum was separated from coagulated blood in sample bottles using bench centrifuge. The plasma and serum samples were harvested by aspiration using Pasteur pipette and transferred into sample bottles.

Fasting blood glucose: Measurement of FPGC was by the glucose oxidase method according to Randox kit manufacturer's procedure (Randox Laboratories Ltd, Ardmore, UK) as previously reported [37].

Serum lipid profile/atherogenic indicators: Evaluation of SLP was according to the methods previously described [37]. Total cholesterol (TC), triacylglycerol (TAG) and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits (Randox Laboratory Ltd., UK). Low-density lipoprotein cholesterol (LDL-C) concentration was estimated according to the formula of Friedewald et al., [40]: LDL-C=TC - (HDL-C)- (TAG/5), as reported by Shaker et al. [41]. Atherogenic Risk Index (ARI) was calculated thus: [TC-(HDL-C)]/(HDL-C) [42] whereas, Castelli Risk Indices (CRIs) were given thus: CRI-I (TC/HDL-C) and CRI-II (LDL-C/HDL-C) [43].

Hepatorenal tissues biomarkers: Measurement of blood hepatorenal function biomarkers, namely, AST/ALT activities, ALP activity, total bilirubin, urea and creatinine concentrations was according the methods previously reported by Ojiako et al. [44].

AST/ALT: Measurement of serum AST and ALT activities were according to the methods of Reitman and Frankel [45].

ALP: Serum ALP activity was assayed by the methods described by Njoku et al. [46].

Total bilirubin: Serum total bilirubin concentration was measured using diazotized sulfanilic acid methods as previously described [47].

Urea: Measurement of serum urea concentration was by the rapid method as described by Fawcett and Scott [48].

Creatinine: Measurement of serum creatinine concentration was according to the methods as described by Bonsnes and Taussky [49].

Statistical analysis

The data collected were analyzed by the ANOVA procedure, while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system package of 9.1 Version in 2006.

Results

GC-MS analysis of AqRE of *C. papaya* showed the presence of seven phytocomponents. The identified phytocomponents with their retention time (RT), molecular formula (M/F), molecular weight (MW) and relative abundance, which was expressed as peak area% (%PA) are summarized in Table 1 and depicted in Figure 1.

S/N	RT (min)	Phytocomponent	M/F	MW	%PA
1	6.121	Dianhydromannitol	$C_6H_{10}O_4$	146	7.36
2	15.695	Methyl-11-hexadecanoate	$C_{17}H_{32}O_2$	268	4.91
3	15.968	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	35.63
4	18.679	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	30.13
5	19.069	Octadecanoic acid, methyl ester	$C_{19}H_{38}O_2$	298	3.49
6	24.669	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethylhexasiloxane	$C_{12}H_{38}O_5Si_6$	430	6.97
7	31.657	Ergosta-5,22-dien-3-ol acetate (3β, 22E)	$C_{30}H_{48}O_2$	440	11.50

RT: retention time; M/F: molecular formula; MW: molecular weight; PA: peak area

Table 1: Phytocomponents detected in AqRE of *C. papaya*.

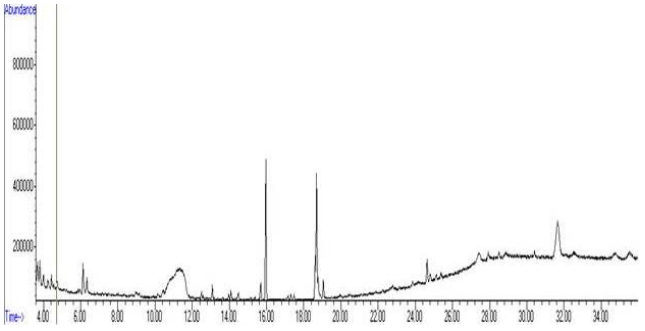

Figure 1: Gas chromatogram of AqRE of *C. papaya*.

The major phytocomponents present in AqRE of *C. papaya*, in terms of their relative abundance, were hexadecanoic acid, methyl ester (35.63%) and 10-octadecenoic acid, methyl ester (30.13%), whereas the minor phytocomponents include ergosta-5,22-dien-3-ol acetate (3β, 22E) (11.50%), dianhydromannitol (7.36%), hexasiloxane-1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl (6.97%), methyl-11-hexadecanoate (4.91%) and octadecanoic acid, methyl ester (3.49%). Table 1 showed that the phytocomponents present in AqRE of *C. papaya* corresponded to 99.99% of the entire GC-MS total ion chromatogram (TIC) (Figure 1).

Additionally, Figure 1 showed that GC-MS total ion chromatogram (TIC) of AqRE of *C. papaya* contained seven phytocomponents with the retention time within the range of 6.121 - 31.657 min. The mass spectra and molecular structures of the identified seven phytocomponents present in AqRE of *C. papaya* are presented in Figures 2A-2G. During the experimental period of 21 days, FBGC of Group 1 varied within the range of 70.75±0.05 - 80.00 ± 0.08 mg/dL; $p>0.05$ (Figure 3). FBGC of Group 2 was significantly higher ($p<0.05$) than that of Group 1 at every 7-day intervals of the experimental

period of 21 days (Figure 3). For instance, FBGC of Group 2 was approximately 3 folds greater than that of Group 1 on days 0, 7 and 14; except on day 21, which gave 2.43 folds; $p<0.05$.

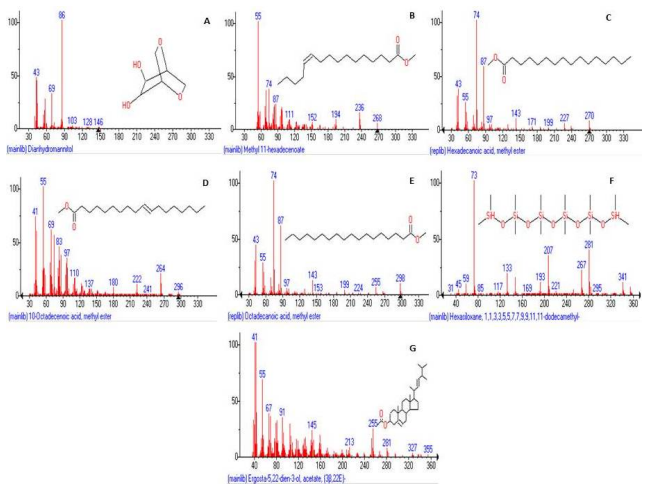

Figure 2: Mass spectra and molecular structures of **(A)** dianhydromannitol, **(B)** methyl-11-hexadecanoate, **(C)** hexadecanoic acid, methyl ester, **(D)** 10-octadecenoic acid, methyl ester, **(E)** octadecanoic acid, methyl ester, **(F)** 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane, **(G)** ergosta-5,22-dien-3-ol acetate (3β, 22E).

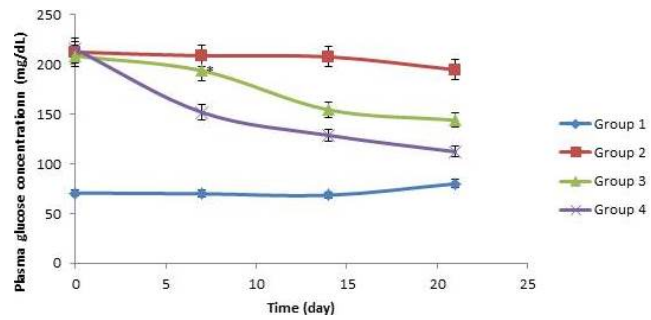

Figure 3: Changes in FPGCs of experimental rat groups; Line plot with asterisk (*) at the given coordinate of point is not significantly different from Group 2 at $p>0.05$ according to LSD.

FBGC of Group 3 was not significantly different ($p>0.05$) from that of Group 2 on days 0 and 7 (Figure 3). However, Figure 3 showed that FBGC of Group 3 was significantly lower ($p<0.05$) than that of Group 2 on days 14 and 21. Specifically, FBGCs of Group 3 on days 14 and 21 represented 25.94% and 30.95% reduction in FBGC in the experimental rats, respectively.

Similarly, FBGC of Group 4 was not significantly different ($p>0.05$) from those of Group 3 and Group 2 (Figure 3). Additionally, FBGC of Group 4 was significantly lower ($p<0.05$) than those of Group 2 and Group 3 on days 7, 14 and 21. Reduction in FBGC of Group 4 on day 21 corresponded to 47.91%. Overall, FBGCs of Group 2, Group 3 and Group 4 were not significantly lower ($p>0.05$) than the normoglycemic benchmark (FPGC<110.0 mg/dL); except that of Group 4 on day 21.

Parameters	Experimental rat groups			
	Group 1	Group 2	Group 3	Group 4
TC (mmol/L)	85.00 ± 0.06^{a}	134.86 ± 0.65^{d}	$97.47 \pm 0.09^{b,c}$	95.00 ± 0.09^{b}
TAG (mmol/L)	29.33 ± 0.05^{a}	66.00 ± 0.07^{d}	$32.61 \pm 0.06^{a,b}$	50.01 ± 0.07^{c}
HDL-C (mmol/L)	$51.66 \pm 0.08^{b,c}$	31.33 ± 0.04^{a}	45.33 ± 0.05^{b}	$53.86 \pm 0.08^{b,c,d}$
LDL-C (mmol/L)	33.03 ± 0.04^{a}	$74.40 \pm 0.06^{c,d}$	70.33 ± 0.05^{c}	45.33 ± 0.05^{b}
VLDL-C (mmol/L)	5.90 ± 0.02^{a}	$15.00 \pm 0.04^{b,c,d}$	$13.03 \pm 0.04^{b,c}$	10.23 ± 0.02^{b}
ARI	0.65	3.3	1.15	0.76
Ratio: LDL-C/HDL-C	0.64	2.38	1.56	0.84
Ratio: TC/HDL-C	1.65	4.31	2.15	1.76
Ratio: TAG/HDL-C	0.57	2.11	0.72	0.93
The mean (X) ± S.D of three (n=3) determinations. Means in the rows with the same letter are not significantly different at $p>0.05$ according to LSD.				

Table 2: SLP of experimental rat groups.

According to reference values, ARI was equivalent to TAG/HDL-C ratio [50,51], TC/HDL-C ratios <1.66 and LDL-C/HDL-C ratio < 1.06 [52]. Castelli Risk Indices (CRIs) I (TC/HDL-C) and II (LDL-C/HDL-C) [43].

Table 2 showed that serum TC concentrations of Group 3 and Group 4 were not significantly different ($p>0.05$) from that of Group 1, whereas serum TC concentration of Group 2 was significantly higher ($p<0.05$) than those of Group 1, Group 3 and Group 4. Similarly, serum TAG concentrations of Group 1 and Group 3 exhibited no significant difference ($p>0.05$). Serum TAG concentration of Group 4 was significantly higher ($p<0.05$) than those of Group 1 and Group 3.

However, serum TAG concentration of Group 4 was significantly lower ($p<0.05$) than that of Group 2 (Table 2).

Table 2 showed that serum HDL-C concentrations of Group 1, Group 3 and Group 4 were comparable but were significantly higher ($p<0.05$) than that of Group 2. Additionally, serum LDL-C concentrations of the experimental rats groups were in the order: Group 2 ≥ Group 3 ($p>0.05$)>Group 4>Group 1. Conversely, serum VLDL-C concentrations of Group 2, Group 3 and Group 4 were comparable but were significantly higher ($p<0.05$) than that of Group 1.

Serum ALT activities of Group 2, Group 3 and Group 4 were profundly elevated compared to Group 1; $p<0.05$ (Figure 4). Conversely, Group 2 exhibited mild elevation in serum AST activity ($p>0.05$). Furthermore, serum AST activities of Group 1, Group 3 and Group 4 were comparable; $p>0.05$.

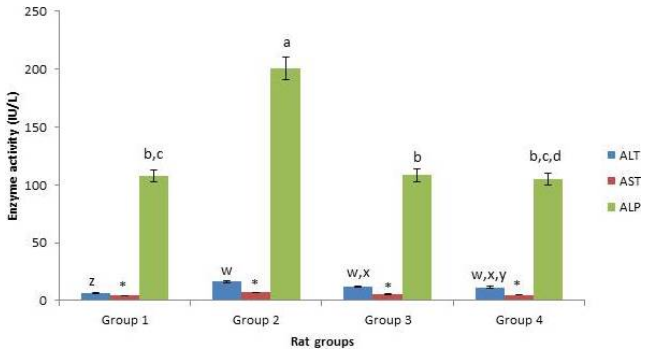

Figure 4: Serum AST, ALT and ALP activities of experimental rat groups. Bars with asterisk (*) are not significantly different from Group 1 at $p>0.05$ according to LSD. For a given parameter, bars with the same letter are not significantly different at $p>0.05$ according to LSD.

Figure 4 showed that serum ALP activity of Group 2 was significantly higher ($p<0.05$) than those of Group 1, Group 3 and Group 4. However, serum ALP activities of Group 1, Group 3 and Group 4 were comparable; $p>0.05$.

Ratios of serum ALT to AST activities were in the order: Group 2>Group 4>Group 3>Group 1 (Table 3). Furthermore, ratios of serum ALT to AST activities of the experimental rat groups were greater than 2 units in Group 2, Group 3 and Group 4

Groups	Group 1	Group 2	Group 3	Group 4
Ratio	1.47	2.32	2.27	2.3

Table 3: Ratios of serum ALT to AST activities of experimental rat groups.

Figure 5 showed that serum total bilirubin concentration of Group 2 was significantly higher ($p<0.05$) than those of Group 1, Group 3 and Group 4. Additionally, serum total bilirubin concentration of Group 1 was not significantly different ($p>0.05$) from that of group 3.

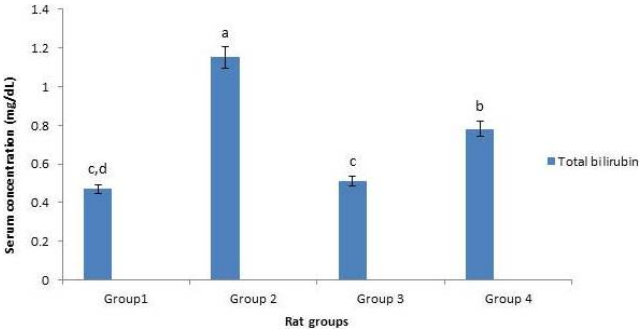

Figure 5: Serum total bilirubin concentrations of experimental rat groups. Bars with the same letter are not significantly different at $p>0.05$ according to LSD.

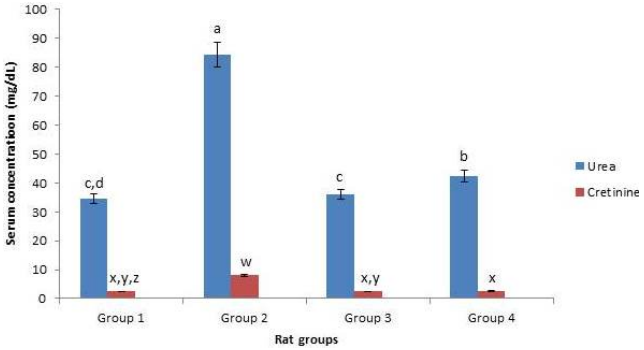

Figure 6: Serum Urea and creatinine concentrations of experimental rat groups. For a given parameter, bars with the same letter are not significantly different at $p>0.05$ according to LSD.

Serum urea concentration of Group 2 was 2.44 folds greater than that of Group 1 (Figure 6). Conversely, serum urea concentration of Group 1 was not significantly different ($p>0.05$) from that of Group 3. Finally, Group 4 gave serum urea concentration that was 1.17 folds greater than that of Group 3; $p<0.05$.

Figure 6 showed that serum creatinine concentrations of Group 1, Group 3 and Group 4 varied within a relatively narrow range of 2.39 ± 0.03 - 2.49 ± 0.02 mg/dL; $p>0.05$. Serum creatinine concentration of Group 2 was significantly higher ($p<0.05$) than those of Group 1, Group 3 and Group 4.

Discussion

GC-MS total ion chromatogram (TIC) of AqRE of *C. papaya* revealed the presence of notable phytochemicals, namely, hexadecanoic acid, methyl ester, 10-octadecenoic acid, methyl ester, ergosta-5,22-dien-3-ol acetate (3β, 22E), dianhydromannitol, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane, methyl-11-hexadecanoate and octadecanoic acid, methyl ester, some of which have been identified and characterized elsewhere according to their therapeutic actions [31,33,53-56]. For instance, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethylhexasiloxane, which is an antibacterial, was identified as a major phytocomponent of n-hexane leaf extract of *C. papaya* variety CO-7 [56]. Fatty acid esters such as

hexadecanoic acid, methyl ester are notable flavour enhancers with antioxidant and hypocholesterolemic activities as well as exhibits inhibitory action on pesticidal 5-α reductase activity [31,33,53-55]. By implication, hexadecanoic acid, which is a product of hexadecanoic acid, methyl ester hydrolysis, exhibits anticancer, anti-microbial, antioxidant and anti-diabetic activity [33]. Additionally, 9,12-octadecadienoic acid (Z,Z)-linoleic acid have been reported to exhibit anti-inflammatory, nematicidal, insecticidal, hypocholesterolemic, cancer preventive, hepatoprotective, anti-histaminic, anti-acne, anti-arthritic, anti-eczemic, 5-α reductase inhibitory action, anti-androgenic and anti-coronary activities [31,57]. The 10-octadecenoic acid, methyl ester from AqRE of *C. papaya* appeared to possess antioxidant activity like 9-octadecenoic acid (Z)-, methyl ester and 9,12-octadecadienoic acid (Z,Z)-, methyl ester from *Croton tiglium* seed as previously described [57].

The present study showed that AqRE of *C. papaya* ameliorated hyperglycemia in the experimental rats within the experimental time of 21 days. Notably, the capacity of AqRE of *C. papaya* to reverse hyperglycemia became effective after day 7 of extract treatment. The present findings have been corroborated by previous studies, in which non-polar extracts from different tissues of *C. papaya* lowered elevated FBGC in chemically-induced diabetic rats [13,28-30]. The 10-octadecenoic acid, methyl ester and hexadecanoic acid, methyl ester, which were major phytocomponents in AqRE of *C. papaya*, are notable antioxidants [31] and might have contributed in ameliorating hyperglycemia in the diabetic rats. According to previous reports, antioxidant phytochemicals are potent anti-diabetic principles [58-61]. Nevertheless, the diabetic rats administered AqRE of *C. papaya* at regular intervals of 7 days for 21 days did not show evidence of full therapeutic benefit, since the FBGC of the rats did not attained the normoglycemic benchmark (FBGC<110 mg/dL).

Evaluation of SLP of experimental rat groups revealed that the untreated diabetic rats showed evidence of dyslipidemia, which is a major complication associated with the DM state [6,62,61]. Accordingly, the untreated diabetic rats exhibited atherogenicity as exemplified by their raised levels of ARI and Castelli Risk Indices (CRIs) I and II. However, the present study showed that AqRE of *C. papaya* ameliorated dyslipidemia in the diabetic rats and were comparable with that of diabetic rats treated with the standard drug-glibenclamide. Previous investigations had reported that 9,12-octadecadienoic acid (Z,Z)- linoleic acid [31] and hexadecanoic acid, methyl ester possessed hypocholesterolemic and anti-coronary activities [63]. Probably by extension, its closely related ester derivatives- 10-octadecenoic acid, and hexadecanoic acid, methyl esters, which were present in AqRE of *C. papaya*, appeared to have exhibited hypocholesterolemic and anti-coronary activities in the diabetic rats. It is worthwhile to note that although atherogenic risk indicators revealed that the diabetic rats treated with AqRE of *C. papaya* showed evidence of recovery from dyslipidemia, ARI and CRIs I and II equally revealed that the diabetic rats did not exhibit full therapeutic benefit within the experimental time.

Serum AST and ALT activities of the untreated and treated diabetic rat groups were such that ALT>AST, which was obvious indication of hepatic tissue necrosis or inflammation and ensuing hepatic dysfunction [64-67] as exemplified in fatty liver disease. Furthermore, the persistent elevation of serum ALT activity over serum AST activity, indicated by the ratios of ALT to AST>2.0 units in the untreated and treated diabetic rat groups, appeared to suggest chemically (alloxan)-induced oxidative damage to hepatic tissues. The present study showed that the diabetic rat groups treated with AqRE of *C. papaya* and glibenclamide exhibited barely adequate recoveries from hepatic tissue injuries within the experimental time. Elevated level of serum ALP activity was diagnostic of obstructive liver disease [68] and level of variation in ALP activity from the reference value defines the extent of damage to hepatic parenchymal cells and micro-obstruction of bile ductules [69]. Serum ALP activities of the diabetic rat groups administered AqRE of *C. papaya* and glibenclamide corroborated previous reports [69,70], in that the pattern of alteration of their serum ALP activity suggested initial distortion in hepatic tissue functional integrity followed by recovery. However, the outcome of the present study showed that the pattern of re-adjustments in serum ALP activities of the diabetic rat groups treated with AqRE of *C. papaya* was not congruent with their corresponding pattern of re-adjustments in serum ALT and AST activities. Additionally, the normalization of elevated serum ALP activity was relatively quicker than that of serum ALT activity in the diabetic rat group following treatment with AqRE of *C. papaya*. These observations were obvious indications that AqRE of *C. papaya* exhibited greater capacity to ameliorate obstructive liver dysfunction than hepatic tissue injuries in the diabetic rats within limits of the experimental time. Overall, the reversion of elevated hepatic tissue dysfunctional indicators of the diabetic rat group, which approached control values, following the administration of AqRE of *C. papaya* may not be unconnected with the actions of antioxidants phytochemicals like hexadecanoic acid, methyl ester and 10-octadecenoic acid, methyl ester present in AqRE of *C. papaya*.

The comparatively elevated serum total bilirubin concentration of untreated diabetic rat group was a further confirmation of hepatic lesion [71,72], which was an indication of a compromised capacity of the hepatocyte to conjugate bilirubin with attendant hyperbilirubinemia [68,73]. Hyperbilirubinemia could also ensue in the absence of liver injury following exacerbated haemolysis as a result of chemically- or pathologically-induced erythrocyte membrane peroxidation. Therefore, elevated serum total bilirubin concentration could also suggest rapid haemolysis as a result of peroxidative damage of the erythrocytes following alloxan intoxication as previously described [74,75]. In human, plasma total bilirubin concentration greater than 1.0 mg/dL is diagnostic of hyperbilirubinemia [68]. The present study showed that the diabetic rat group treated with AqRE of *C. papaya* did not exhibit blood index (serum total bilirubin concentration>1.0 mg/dL) that was diagnostic of hyperbilirubinemia. The comparable serum total bilirubin concentration of the normal rat group with that of the diabetic rat group treated with AqRE of *C. papaya* may not be unconnected with hepatoprotective and erythrocyte membrane stabilization potentials of antioxidant phytocomponents present in *C. papaya* extract as previously reported [31,57,76].

Urea and creatinine are blood low threshold substances and as such, are rapidly clear from circulation by the kidney. Metabolic studies revealed that urea is a nitrogenous waste product of dietary origin, whereas creatinine is, for the most part, derived from muscle protein turnover [77,78]. The rapidity with which urea and creatinine are cleared from circulation and excreted in the urine is a diagnostic parameter for ascertaining renal tissue functionality. The present study conformed to previous reports, in which streptozotocin-induced diabetic rats and Wistar rats fed with unprocessed cocoa exhibited marginal elevation of serum creatinine concentration but with marked elevation of serum urea concentration, which was diagnostic of renal dysfunction [79,80]. However, studies showed that elevated serum creatinine concentration was a more reliable diagnostic biomarker

than that of serum urea concentration for renal dysfunction [81]. Serum creatinine concentration of the diabetic rat groups treated with AqRE of *C. papaya* and glibenclamide were comparable with the normal rat group. Furthermore, serum urea concentration of diabetic rat groups treated with AqRE of *C. papaya* parallel that of the normal rat group. These findings were obvious indications of the capability of antioxidant phytocomponents present in AqRE of *C. papaya*, notably, hexadecanoic acid, methyl ester and 10-octadecenoic acid, methyl ester, to reverse renal dysfunction in alloxan-induced diabetic rats. Earlier studies corroborated the present findings, in which extracts of *Diospyros lotus* and *Cnestis ferruginea* exhibited renal protective activity in animal models, which was largely due to the presence of antioxidant phytochemicals in the extracts [82,83]. Additionally, a summary of some medicinal plants with nephroprotective activity has been reported elsewhere [84].

Conclusion

GC-MS chromatogram of AqRE of *C. papaya* revealed the presence of phytochemicals of established diverse therapeutic potentials. Accordingly, the phytocomponents present in AqRE of *C. papaya* ameliorated hyperglycemia in alloxan-induced diabetic rats. Likewise, ARI and CRIs I and II revealed that the AqRE of *C. papaya* treated diabetic rat group showed evidence of recovery from dyslipidemia. Also, the diabetic rat group treated with AqRE of *C. papaya* exhibited marginal recovery from hepatic tissue injuries. Furthermore, blood renal function indicators showed that AqRE of *C. papaya* reversed renal dysfunction in the diabetic rat group. The limited therapeutic benefit of the diabetic rat group treated with AqRE of *C. papaya* was probably as a result of the nature of extract and mode with which the extract was administered to the diabetic rats. Therefore, in order to ascertain the possibility of improving the efficacy of root extract of *C. papaya*, a corresponding experiment should be carried out using organic/polar extract of *C. papaya* as well as increasing the frequency of intermittent treatments of the diabetic rats. Finally, we recommend further investigations, in this regard, using isolated individual phytocomponents present in AqRE of *C. papaya* identified in the present study.

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