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Evaluation of the Effects of *Citrus paradisi* (Rutaceae) Fruit Juice on Electrolyte, Hepatic, Haematological and Histological Derangements in Streptozotocin-Induced Diabetic rats.

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ABSTRACT

This study investigated the effects of *Citrus paradisi* fruit juice on biochemical, haematological and histopathological indices in streptozotocin-induced diabetic rats. Wistar rats of either sex (180-300 g) were fasted overnight and diabetes mellitus induced using streptozotocin 40 mg/kg IP. Diabetes mellitus (fasting blood glucose ≥ 200 mg/dl) was confirmed 48 hours later. The rats were randomly grouped into six (n = 5). Fourteen days later, the rats were sacrificed under chloroform anaesthesia. Vital organs (kidneys, liver and pancreas) and blood samples were obtained for histopathological, biochemical and haematological analysis. *Citrus paradisi* (500 mg/kg and 1000 mg/kg) caused significant ($p < 0.05$) elevations in red blood cell counts and red blood cell indices. *Citrus paradisi* (250 mg/kg, 500 mg/kg and 1000 mg/kg) caused significant ($p < 0.05$) reductions in white blood cell and platelet counts but did not significantly affect the differential white blood cell count. Liver transaminases and total bilirubin were reduced significantly ($p < 0.05$) while the total protein was significantly increased ($p < 0.05$). *Citrus paradisi* (500 mg/kg and 1000 mg/kg) brought about significant improvements in the histopathological indices in diabetic rats. These data suggest that *Citrus paradisi* ameliorates biochemical, haematological and histopathological changes associates with diabetes mellitus.

Key words: Diabetes; *Citrus paradisi*; Hematological; Histopathological; Biochemical; Streptozotocin

INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by persistent hyperglycemia (Neelesh *et al.*, 2010) due to defects in insulin secretion and/or action (Maritim *et al.*, 2003). Statistics from the International Diabetes Federation show that diabetes mellitus affects about 366 million people on a global scale with the figure likely to increase to 552 million or greater by the year 2030 (Whiting *et al.*, 2011). In Africa, over 14 million people corresponding to about 4.3% of adults have diabetes mellitus and this was responsible for 401276 deaths in the continent (IDF,2012). West Africa recorded the highest cases with Nigeria and Cote D'ivoire having 3.2 million and 401123 diabetics respectively. An array of complications could occur due to poorly treated or untreated diabetes. These include nephropathy, metabolic syndrome, electrolyte derangements, hematological alterations and hepatic dysfunction (Oyedemi *et al.*, 2011; Kotharia and Bokariya, 2012). Proper and timely interventions can avert these complications. Drug and non-drug measures are used for treatment. Drugs used include insulin, amylin analogues, insulin secretagogues such as sulfonylureas, meglitinides and incretin mimetics, insulin sensitizers such as biguanides and thiazolidinediones, alpha glucosidase inhibitors, inhibitors of sodium glucose co-transporter 2, dopamine agonists and bile acid resins (Powers and D'Alessio, 2011). Non-pharmacological measures include dietary and lifestyle modifications. Diabetes remains a public health concern. This is due to various factors: The side effects of the medications. Such side effects include hypoglycemia, lactic acidosis, weight gain and pancreatitis. The cost of the drugs is also a concern (Akinmoladun and Akinloye, 2007). *Citrus paradisi* Macfad (of the family Rutaceae) fruit known as Grapefruit (English), *lemu* (Hausa) and *oson lemu* (Yoruba) in some Nigerian languages comes in varieties including white, purple and pink as determined by genetics. It has a glycemic index of 25 which makes it a good option for diabetics as a glycemic index of less than 55 is considered low and appropriate for diabetic patients (Gate, 2017). In Nigerian folkloric medicine, it finds use in managing diabetes (Mohammed *et al.*, 2014). The fruit is washed and the pulp obtained. The pulp is then juiced or blended and the juice filtered. The filtered juice is then consumed (Mohammed *et al.*, 2014). Streptozotocin is a chemical model for inducing diabetes mellitus in laboratory animals. It is effective in many animal species and is reproducible (Joo *et al.*, 2010).

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It produces many of the acute and chronic complications of human diabetes hence, it is an appropriate model to assess the mechanism of diabetes. *Citrus paradisi* enhances appetite and is employed for its digestive, stomachic, antiseptic and diuretic properties (Herbal medicine, 2000). *Citrus paradisi* is used in aromatherapy due to its aromatic scent (Herbal medicine, 2000). The hypoglycaemic effect of *Citrus paradisi* seed extract has been reviewed by Adeneye (2008). The antidiabetic effects of the juice have been studied by Chukwuma *et al* (2016). *Citrus paradisi* contains phytochemicals including flavonoids, tannins, alkaloids, limonoids and lycopene. It is also a rich source of vitamin C, dietary fiber, vitamin A, potassium, folate and vitamin B5. It also contains high concentrations of iron, calcium and other minerals. Pink and red varieties of grapefruits are an excellent source of beta carotene, high in fiber and have a low caloric level. Some studies evaluating the antidiabetic effects of *Citrus paradisi* have been carried out. However, these have been done largely on the pink, red and purple varieties of the fruit. No study has provided information on the effects of *Citrus paradisi* on electrolyte derangements, some hepatic and hematological indices as well as histopathological indices in a streptozotocin-induced type-2 diabetes model hence this study.

MATERIALS AND METHODS

Materials, Drugs and Chemicals

Sodium citrate (JHD, China), citric acid (JHD, China), buffers 4 and 7 (Loba Chem, India), distilled water, streptozotocin (Santa Cruz Biotechnology, USA), methylated spirit (Nomagbon Pharmaceutical Industries, Nigeria), glibenclamide (Sigma Aldrich, USA), chloroform (Fharmtrends Nigeria limited, Nigeria), 1 % hydrochloric acid (BDH chemicals, England), neutral buffer formalin (Thermo Scientific, USA), 70 and 96 % ethanol (Fharmtrends Nigeria limited, Nigeria), haematoxylin-eosin dye (BDH Chemicals, England), Hetich centrifuge (Rotofix 32A, Germany), human automated haematology system analyzer (ERMA PCE 210, ERMA, Japan), optical photomicroscope (Leica MC170HD Biosystems, Germany), measuring cylinder, test tubes, beaker, spatula, crucible, plastic cages, 1 ml syringes, 2 ml syringes, 5 ml syringes, surgical dissecting kits, plain sample bottles, EDTA bottles, cotton wool, surgical gloves, weighing balance, electric burner, orogastric tube, Chiffon bag.

Animals and Ethical Approval

Wistar rats of either sex (180 - 300 g) were purchased from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin city, Edo state, Nigeria. They were left to acclimatize for a period of two weeks and fed standard pellet feed (Top feed finisher pellet, Ibadan, Nigeria) and water *ad libitum.* High sanitary and normal lighting conditions were maintained. Experimental protocols were carried out in accordance with the National Institute of Health Guidelines for the Care and Use of laboratory animals (NIH Publication No. 80-23) revised in 2002. Ethical approval was granted by the Research and Ethics Committee of the Faculty of Pharmacy, University of Benin with the reference number: EC/FP/017/04.

Plant Collection, Authentication and Extraction

Citrus paradisi was purchased from a grocery store and botanical garden along airport road, Benin city, Midwestern Nigeria. This was identified in the National Institute for Pharmaceutical Research and Development, Abuja with herbarium voucher number NIPRD/H/7056 issued. Freshly purchased *Citrus paradisi* fruits were thoroughly washed, cut into quarters and the fruit pulp obtained. The pulp from the fruit was homogenized with the aid of a blender and filtered using a chiffon bag to obtain the pure juice. The percent yield was determined by dividing the weight of the filtered juice by the weight of the blended pulp and multiplying by 100. The filtrate was stored in the deep freezer at -20ºC until needed.

Determination of the Extractive Value for *Citrus paradisi* Fruit Juice

This was done to determine the appropriate volume of the juice to be given to the rats during the investigations. The concentration of the juice was determined by evaporating 5 ml of the juice in a crucible of a known weight using an electric burner at 70˚C. The crucible containing the residue was allowed to cool and weighed. The weight of the residue was gotten by subtracting the weight of the crucible from the weight of the crucible and residue. This process was repeated two times and the extractive value determined (Chukwuma et al., 2016). The extractive value was 72 mg/ml.

Phytochemical Analysis

Phytochemical analysis was performed using standard procedures as given by Sofowora (2008) and Evans (2002) as follows:

Test for carbohydrates: 3ml of *Citrus paradisi* was added to a few drops of α- naphthol solution in

alcohol and shaken after which a few drops of Conc. H_2SO_4 were added. A violet ring formed at the junction of the two liquids indicated the presence of carbohydrate.

Test for Saponins: 3 ml of *Citrus paradisi* was shaken vigorously in a test tube after which it was observed for 5 minutes. Persistent frothing indicated the presence of saponins.

Test for Anthraquinones: 3 ml of *Citrus paradisi* was shaken with 10 ml chloroform, filtered and 5 ml of 10% ammonia solution added to the filtrate and the mixture shaken properly. The presence of a pink coloration indicated the presence of anthraquinones.

Test for Flavonoids: 3 ml of *Citrus paradisi* was defatted with acetone, filtered and the residue extracted with water on a water bath. This was filtered again. After the second filtration, 10 % Lead acetate solution was added to 5 ml of the filtrate while 5 ml of 10 % sodium hydroxide was added to an equal volume of the filtrate. The presence of a yellow coloration indicated the presence of flavonoids.

Test for Tannins: 3 ml of *Citrus paradisi* was boiled for 5 minutes and then cooled after which it was filtered and ferric chloride solution added to 2 ml of the filtrate in a test tube. A brown precipitate indicated the presence of tannins.

Test for Alkaloids: A few drops of dilute hydrochloric acid were added to 2 ml of *Citrus paradisi* fruit juice. This was boiled in a water bath for 30 minutes and filtered. The filtrate was tested for alkaloids using Dragendoff's, Wagner's and Hagar's reagents.

Acute Toxicity Study

Acute toxicity studies of *Citrus paradisi* was performed using a modification of Lorke's method (Lorke, 1983). In phase 1, nine (9) rats were randomly divided into three groups of three rats. The rats in the three groups were administered with the aid of an orogastric tube, single doses (10, 100 and 1000 mg/kg body weight respectively) of the *Citrus paradisi* juice orally. These were observed for signs of toxicity. In the absence of toxicity, the second phase was carried out.

In Phase 2, based on the results of the phase 1 study, three rats (One rat per group) were orally administered single doses of 1600, 2,900 and 5,000 mg/kg body weight of *Citrus paradisi*. The rats were kept under the same conditions and observed for signs of toxicity and mortality for 24 hours. The LD_{50} was calculated based on the results as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses where 0 and 100 % survival rates were recorded. Where the extracts at doses above 5000 mg/kg did not cause any visible toxicity or mortality, the extracts were considered safe. The control group was administered 1 ml distilled water orally. All the rats used for the acute toxicity study were observed daily for any signs of toxicity over a period of two weeks.

Diabetes Induction

Thirty rats were fasted over-night and diabetes mellitus was induced using a single intra-peritoneal dose of streptozotocin (40 mg/kg body weight) dissolved in freshly prepared 0.1 M citrate buffer pH 4.5.

The rats were provided with a 5 % w/v solution of glucose to prevent streptozotocin-induced hypoglycaemia seen within the first few hours of induction (Eleazu *et al.,* 2013). The rats had free access to feed after the administration of streptozotocin. Forty-eight hours later, diabetes was confirmed using a blood glucose level of $\geq$ 200 mg/dl as a criterion (Rheney and Kirk, 2000). Blood glucose levels were measured with the aid of Accu-Chek® Active glucometer (Roche, USA).

Experimental Protocol

The experiments were executed at the postgraduate laboratories of the Department of Pharmacology and Toxicology, University of Benin, Nigeria. A modification of the method of Chukwuma *et al* (2016) was used for daily treatment. The diabetic rats were split into six groups of five rats each (n = 5) depending on the intervention. Daily treatment was carried out for two weeks as follows:

Diabetic rats treated with 250 mg/kg *Citrus paradisi* fruit juice

Diabetic rats treated with 500 mg/kg *Citrus paradisi* fruit juice

Diabetic rats treated with 1000 mg/kg *Citrus paradisi* fruit juice

Diabetic rats receiving 1 ml distilled water (untreated diabetic control)

Diabetic rats treated with 5 mg/kg glibenclamide (Standard drug control) using 1 ml distilled water as vehicle.

Non-diabetic rats receiving 1 ml distilled water (Normoglycemic control)

Body Weight Change Determination

Diabetic rats were weighed weekly using a sensitive weighing balance and weight changes determined

Haematological and Biochemical Endpoint Determination

Following two weeks treatment, animals were anesthetized with chloroform, blood taken from the abdominal aorta into edetate and plain sample bottles and taken to the laboratory and analyzed in the chemical pathology and haematology laboratories of the University of Benin Teaching Hospital. The following end-points were determined:

Assay for haematological indices: Blood samples in the EDTA bottles were injected into the chamber of the human automated haematology system analyzer and diluted with an isotonic solution of saline. Indices analyzed included haemoglobin, red blood cell count, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin concentration, mean corpuscular haemoglobin, platelet count, white blood cell count and differential white blood cell count.

Assay for alanine amino transferase: This test was carried out by measuring the concentration of pyruvate hydrazone formed by 2,4-dinitrophenylhydrazine. 1 ml of reagent 1 (sample blank) was added to 200 µl of serum in a sample test tube. They were mixed and incubated for 5 minutes at 37˚C. 1 ml of reagent 2 (standard) and 200 µl of serum sample were added to the sample blank test tube as well as the sample test tube. The solutions in the test tubes were mixed and allowed to stand for 20 minutes at 25˚C. 5 ml of sodium hydroxide was added to the test tubes. The solutions in the test tubes were mixed. The absorbance of the sample against the sample blank was measured at 54 nm after 5 minutes. The concentration of the sample was obtained from the absorbance values according to manufacturer's specifications (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963).

Assay for aspartate amino transaminase: Same as alanine amino transferase

Assay for alkaline phosphatase: To the respective test tubes, 50 µl of standard, control and serum samples were added at intervals. These solutions were gently mixed. De-ionized water served as blank. The solutions were incubated for 10 minutes at 37˚C. 2.5 ml of alkaline phosphatase colour developer was added to the test tubes at intervals. These were mixed and the absorbances were measured at 590 nm. Alkaline phosphatase was calculated as:

Alkaline phosphatase (IU/L) = Absorbance of sample/Absorbance of standard x 50

Assay for electrolytes (Sodium, chloride, bicarbonate and potassium): 0.01 N HCl was added to 200 µl of serum sample. The solution was mixed and 1 drop of phenol red indicator added. The mixture was titrated with 0.01 N sodium hydroxide to obtain a brick red colour which serves as endpoint (Van Skye and Neil, 1924). Bicarbonate was calculated as thus:

Bicarbonate (µmol/l) = 50 – Titre

Titre = Endpoint x 100

The assay for chloride exploits the formation of chloride precipitate in a sample using mercuric nitrate. When chloride is titrated with standard solution of mercuric ion, undissociated but stable mercuric chloride is formed. The excess mercuric chloride nitrate reacts with diphenylcarbazone to produce a violet coloration. 2 ml of deionized water was added to 200 µl of the serum sample. This solution was mixed thoroughly and 3 drops of diphenylcarbazone indicator and 1 drop of nitric acid were added. The mixture was titrated with mercuric nitrate to give a violet endpoint. The same procedure was repeated for chloride standard solution (Schales and Schales, 1941). Chloride was calculated as thus:

Chloride (µmol/l) = Titre of sample/Title of standard x 100

The assay method for sodium and potassium entailed the injection of solutions containing these elements in flame leaving solid salt, which dissociated at neutral ground state. The atoms were made excited in the flame, thus moving to a higher energy state. The excited atoms then fell back to ground state emitting light of characteristic wavelength (590 nm for sodium and 770 nm for potassium). The light was passed through a suitable filter onto a photosensitive element and the amount of current measured was proportional to the amount of sodium and potassium present in the serum sample (Magoshes and Vallee, 1956)

Assay for bilirubin: To the blank test tube, 0.2 ml of sulphanilic acid, 1 ml of caffeine and 0.2 ml of serum were added. 0.2 ml of sulphanilic acid, 1 drop of sodium nitrite, 1 ml of caffeine and 0.2 ml of serum were added to the sample test tube. The solutions in the test tubes were mixed and allowed to stand for 10 minutes at room temperature. 1 ml of tartrate was added to the test tubes. The solutions in the test tubes were mixed and allowed to stand for 5 minutes at room temperature. The absorbances were measured at 578 nm (Schmidt and Schmidt, 1963). Total bilirubin was calculated as thus:

Total bilirubin (mg/dl) = Absorbance of sample x 10.8

To determine conjugated bilirubin, 0.2 ml of sulphanilic acid, 2 ml of saline and 0.2 ml of serum were added to the blank test tube.0.2 ml of sulphanilic acid, 1 drop of sodium nitrite, 2 ml of saline and 0.2 ml of serum were added to the sample test tube. The solutions in the test tubes were mixed and allowed to stand for 5 minutes at room temperature. The absorbance was measured at 546 nm (Schmidt and Schmidt, 1963). Conjugated bilirubin was calculated as thus:

Conjugated bilirubin (mg/dl) = Absorbance of sample x 14.4

Assay for proteins: To the reagent blank test tubes, 0.02 ml of distilled water and 1 ml of reagent 1(blank) were added. 0.02 ml of serum and 1 ml of reagent 1 were added to the sample test tubes. 0.02 ml of standard and 1 ml reagent 1 were added to the standard test tubes. The solutions in the test tubes were mixed and incubated at 25˚C for 30 minutes. The absorbance of the sample and the standard against the reagent blank was measured at 546 nm (Tietz, 1995). Total protein was calculated as thus:

Total protein (g/dl) = 19 x Absorbance of sample

To the reagent blank test tube, 10 µl of distilled water and 3000 µl of reagent 1 were added. 10 µl of standard and 3000 µl of reagent 1 were added to the standard test tube. 10 µl of serum and 3000 µl of reagent 1 were added to the sample test tube. The solutions in the test tube were mixed and incubated for 5 minutes at 25˚C. The absorbances of the samples and standard were measured against the reagent blank at 630 nm (Doumas *et al.*, 1971; Grant *et al.*, 1987). Albumin was calculated as thus:

Albumin (g/dl) = Absorbance of sample/Absorbance of standard x 4.5

Globulin = Total protein – Albumin.

Histopathology

The liver, kidneys and pancreas were excised from chloroform anesthetized rats and fixed in neutral buffered formalin fixative. The fixed organs were dehydrated using absolute ethanol followed by 96% ethanol, 70% ethanol and then rinsed with distilled water. A 4 µm section was prepared for each organ. Staining using haematoxylin-eosin dye was performed and the stained tissues viewed by a histopathology expert in the histopathology unit of the University of Benin Teaching Hospital using an optical photomicroscope (Leica MC170 HD, LeicaBiosystems, Germany) at a magnification power of x 400.

Statistical Analysis of Data

All data were expressed as mean ± standard error of mean (SEM). Analysis was performed using one-way ANOVA followed by Dunnett's post-hoc test (Graphpad Prism® San Diego, USA). $P < 0.05$ was considered significant.

RESULTS

Properties of Citrus Juice and Phytochemical Analysis

Citrus paradisi fruit juice was a yellowish liquid with an astringent taste. It had a yield of 21.4% and an extractive value of 72 mg/ml.

Phytochemical analysis of *Citrus paradisi* using chemical tests revealed that the juice contained carbohydrates, alkaloids, flavonoids, tannins, saponins and anthraquinones. Glycosides were absent

Acute Toxicity Study

No mortality was observed when *Citrus paradisi* was administered even at doses as high as 5000 mg/kg. No mortality was also reported over the two-week observation period

Effects of *Citrus paradisi* on Weight Changes

The weights of rats treated with *Citrus paradisi* (250 mg/kg, 500 mg/kg and 1000 mg/kg) were significantly less than the normal control at the end of week 1. Remarkable progress was seen at the end of week 2 as the weights of rats treated with *Citrus paradisi* (500 mg/kg and 1000 mg/kg) showed no significant difference from the normal control (Table 1).

Effects of *Citrus paradisi* on Hematological Parameters

Citrus paradisi significantly ($p < 0.05$) elevated the erythrocytes when compared to the diabetic control. *Citrus paradisi* (250 mg/kg) however, showed no marked increase. Significant increases ($p < 0.05$) in hemoglobin concentration relative to the diabetic control were seen. *Citrus paradisi* (250 mg/kg) however, showed no increase of significance. *Citrus paradisi* (500 mg/kg and 1000 mg/kg) showed increases ($p < 0.05$) in haematocrit when compared to the diabetic control. *Citrus paradisi* (1000 mg/kg) significant ($p < 0.05$) increased the mean cell hemoglobin concentration over values seen with the diabetic untreated control. The various doses of *Citrus paradisi* significantly ($p < 0.05$) decreased leucocyte and thrombocyte counts in comparison to diabetic controls. The differential leucocyte count and the mean cell volume did not significantly change in comparison to the diabetic control (Tables 2 and 3).

Effects of *Citrus paradisi* on Serum Biochemistry

Citrus paradisi increased bicarbonate and sodium levels while decreasing potassium levels. Chloride levels were unchanged. *Citrus paradisi* juice caused significant ($p < 0.05$) decrements in liver transaminases in comparison to the diabetic untreated control. Various doses of the juice except *Citrus paradisi* (250 mg/kg) significantly ($p < 0.05$) increased serum total proteins. Treatment with *Citrus paradisi* (1000 mg/kg) caused a significant increase

($p < 0.05$) in serum albumin but not globulin levels. The juice except *Citrus paradisi* (250 mg/kg) significantly ($p < 0.05$) decreased total bilirubin. Direct bilirubin levels were unchanged across the board (Tables 4 to 6).

Effects of *Citrus paradisi* on Histopathology

Liver (Figure 1):

Normal liver section shows a prominent histological feature showing portal vein (arrow) with well fenestrated sinusoids and hepatocytes with distinct nucleus. Diabetic control liver section reveals prominent histological feature showing portal vein (arrow) slightly congested and scanty inflammation surrounding it with visible fatty hydropic changes (arrow head). Liver of rat treated with *Citrus paradisi* (250 mg/kg) depicts central vein (long arrow) and radiating hepatocytes (arrow head) with a nucleus which is not distinct. Liver of rat treated with *Citrus paradisi* (500 mg/kg) shows prominent hepatocytes with a visible nucleus (arrow head) and a prominent portal vein (long arrow) surrounded by mild inflammatory cells (short arrow). Liver of the rat treated with *Citrus paradisi* (1000 mg/kg) appears distinct with well fenestrated sinusoidal space and hepatocytes with pyknotic nucleus (arrow head). There is mild inflammation around the central vein (short arrow). Liver of rat treated with glibenclamide (5 mg/kg) reveals prominent histological feature showing portal vein (long arrow) with mild fatty nucleus changes (short arrow).

Kidney (Figure 2): Normal kidney section depicts normal histological features with detailed cortical parenchyma and the renal corpuscles appearing as dense rounded structures (arrow). Diabetic control kidney section reveals detailed cortical parenchyma with the renal corpuscles appearing as atrophied dense rounded structures (arrow) bounded by noteworthy inflammatory infiltrates (arrow head). Kidney of rat treated with *Citrus paradisi* (250 mg/kg) depicts a kidney characterized by an atrophied glomerulus with clearing to the corpuscles (long arrow) and mild focal tubular necrosis (short arrow). Kidney of rat treated *Citrus paradsi* (500 mg/kg) reveals cortical parenchyma and tubules (short arrow). The renal corpuscles appear as dense rounded enlarged structures (long arrow). Kidney of rat treated with *Citrus paradisi* (1000 mg/kg) shows cortical parenchyma and tubules (short arrow) and the renal corpuscles appearing as dense rounded enlarged structures with mild inflammatory changes (long arrow). Kidney section of rat treated with glibenclamide (5 mg/kg) shows normal histological features with detailed cortical parenchyma (short arrow) and the renal corpuscles appearing as dense rounded structures (long arrow). Pancreas (Figure 3): Pancreas of normal control shows an acinar pattern structure with the nuclei of some of the acinar cells appearing. The acinar cells stained strongly and are arranged in lobules with prominent nuclei. The islet cells are embedded within the acinar cells and surrounded by a fine capsule (arrow head). Pancreas of untreated diabetic control reveals some acinar cells with islet cells showing congestion and pyknosis of the nuclei with visible lymphocytic infiltrates (arrow). Pancreas of rat treated with *Citrus paradisi* (250 mg/kg) reveals secretory acini (long arrow) with mild atrophy of the pancreatic islet and a pyknotic nuclei. Some acinar cells with islet cells show congestion with visible lymphocytic infiltrates (short arrow). Pancreas of rat treated with *Citrus paradisi* (500 mg/kg) reveals prominent secretory acini arranged in lobules. Pancreas of rat treated with *Citrus paradisi* (1000 mg/kg) reveals some acinar cells with congestion of the pancreatic islet cells. Pyknotic nuclei are seen (long arrow). Reduced lymphocytic infiltration is seen (arrow head). Pancreas of rat treated with glibenclamide (5 mg/kg) reveals acinar pattern structure with pyknotic nuclei of some acinar cells appearing. The acinar cells which stained strongly are arranged in lobules with prominent nuclei (long arrow).

Table 1: Effects of *Citrus paradisi* on weight (n = 5)

	Week 0	Week 1	Week 2
Normal	209.0±4.68	213.6±5.73	212.0±2.25
Untreated	223.0±7.78	204.1±9.40[a]	196.8±8.68[a]
Glibenclamide 5 mg/kg	217.5±4.59	231.4±4.26	236.8±3.96
Citrus paradisi 250 mg/kg	197.4±3.44	181.0±6.68[a]	177.0±5.66[a]
Citrus paradisi 500 mg/kg	201.5±5.48	204.6±5.12[a]	209.8±4.65
Citrus paradisi 1000 mg/kg	194.9±2.61	200.0±2.09[a]	204.9±1.86

Normal: Normal control, Untreated: Untreated diabetic control. [a] weight significantly less than normal at $p < 0.05$. Results are expressed as mean ± SEM

Table 2: Effects of *Citrus paradisi* on red blood cell parameters (n = 5)

	RBC (x 10^6/ul)	HB (g/dl)	HCT (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Normal	8.86±0.22	13.64±0.54	44.18±0.71	50.20±1.20	16.39±0.25	30.87±1.29
Untreated	7.00±0.25[b]	9.38±0.36[b]	39.02±1.00[b]	51.50±3.00	13.40±0.72[b]	24.03±0.31[b]
Glibenclamide 5 mg/kg	8.57±0.17[a]	14.30±0.21[a]	43.90±0.77[a]	52.00±1.00	16.68±0.15[a]	33.25±0.50[a]
C.paradisi 250 mg/kg	7.99±0.19	10.38±0.34	41.98±0.29	51.00±0.60	12.99±0.69	24.70±1.00
C.paradisi 500 mg/kg	8.04±0.29[a]	11.94±0.40[a]	44.38±0.51[a]	51.80±0.84	14.85±0.72[a]	27.00±0.90[a]
C.paradisi 1000 mg/kg	8.55±0.47[a]	12.72±0.48[a]	44.10±0.90[a]	51.00±1.00	14.87±0.59[a]	28.80±0.91[a]

RBC: Red Blood Cell, HB: Haemoglobin, HCT: Haematocrit, MCV: Mean Cell Volume, MCH: Mean Cell Haemoglobin, MCHC: Mean Cell Haemoglobin Concentration, Normal: Normal control, Untreated: Untreated diabetic control, *C.paradisi: Citrus paradisi*. [a] $p < 0.05$ relative to diabetic control, [b] $p < 0.05$ relative to normal control. Results are expressed as mean ± SEM

Table 3: Effects of *Citrus paradisi* on white blood cell parameters and platelet count (n = 5)

	WBC (x10^3/ul)	Lymphocytes (%)	Granulocytes (%)	Monocytes (%)	Platelets (x10^3/ul)
Normal	5.92±0.31	50.28±2.12	48.24±3.34	1.48±0.18	228.8±12.00
Untreated	14.92±0.82[b]	48.31±5.51	49.79±1.61	1.90±0.66	700.0±27.84[b]
Glibenclamide 5 mg/kg	7.28±0.14[a]	51.20±3.20	47.42±3.17	1.38±0.24	283.8±19.63[a]
C.paradisi 250 mg/kg	9.04±0.31[a]	48.21±1.65	50.01±2.28	1.79±0.22	403.3±46.98[a]
C.paradisi 500 mg/kg	7.68±0.47[a]	50.28±2.06	48.10±3.56	1.62±0.34	360.4±35.90[a]
C.paradisi 1000 mg/kg	6.42±0.61[a]	49.30±2.65	49.66±2.32	1.04±0.34	341.8±43.32[a]

WBC: White Blood Cell, Normal: Normal control, Untreated: Untreated diabetic control, *C.paradisi: Citrus paradisi.* [a] $p < 0.05$ relative to diabetic control, [b] $p < 0.05$ relative to normal control. Results are expressed as mean ± SEM

Table 4: Effects of *Citrus paradisi* on Electrolytes (n = 5)

	Bicarbonate (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)
Normal	26.00 ± 1.23	142.2±0.86	4.82±0.32	100.2±0.37
Untreated	12.60±0.68[b]	120.5±1.04[b]	9.40±0.26[b]	103.0±0.45
Glibenclamide 5mg/kg	23.40±0.75[a]	141.8±1.16[a]	5.76±0.24[a]	102.4±1.29
C. paradisi 250 mg/kg	21.40±0.93[a]	137.6±2.68[a]	7.38±4.34[a]	102.4±1.28
C. paradisi 400 mg/kg	22.40±0.51[a]	141.2±2.33[a]	6.02±0.30[a]	102.0±0.70
Citrus paradisi 800 mg/kg	24.80±1.46[a]	139.2±1.43[a]	6.02±0.27[a]	102.4±0.40

Normal: Normal control, Untreated: Untreated diabetic control, *C.paradisi: Citrus paradisi.* [a] $p < 0.05$ relative to diabetic control, [b] $p < 0.05$ relative to normal control. Results are expressed as mean ± SEM

Table 5: Effects of *Citrus paradisi* on Serum Liver Enzymes (n = 5)

	ALP (μ/L)	AST (μ/L)	ALT (μ/L)
Normal	106.2±1.02	98.20±2.71	57.20±1.59
Untreated	811.2±34.36[b]	160.0±3.26[b]	152.4±15.76[b]
Glibenclamide 5 mg/kg	216.2±6.83[a]	122.6±2.23[a]	97.80±1.77[a]
C.paradisi 250 mg/kg	345.2±23.64[a]	141.2±4.42[a]	119.4±7.94[a]
C.paradisi 500 mg/kg	208.2±10.76[a]	114.0±7.98[a]	75.80±8.15[a]
C.paradisi 1000 mg/kg	206.2±9.67[a]	106.8±4.41[a]	68.40±7.89[a]

ALP: Alkaline Phosphatase, AST: Aspartate Transaminase, ALT: Alanine Transaminase, Normal: Normal control, Untreated: Untreated diabetic control, *C.paradisi: Citrus paradisi.* [a] $p < 0.05$ relative to diabetic control, [b] $p < 0.05$ relative to normal control. Values are expressed as Mean±SEM

Table 6: Effects of *Citrus 8aradise* on Serum Protein and Bilirubin Levels (n = 5)

	TP (mg/dl)	ALB (mg/dl)	GLO (mg/dl)	TB (mg/dl)	DB (mg/dl)
Normal	7.52±0.18	4.44±0.31	3.08±0.23	0.28±0.02	0.12±0.02
Untreated	5.70±0.18[b]	3.06±0.21[b]	2.64±0.25	0.48±0.02[b]	0.22±0.04
Glibenclamide 5 mg/kg	6.90±0.05[a]	4.46±0.21[a]	2.44±0.33	0.28±0.02[a]	0.12±0.02
C.paradisi 250 mg/kg	6.30±0.18	3.68±0.23	3.28±0.28	0.40±0.04	0.22±0.14
C.paradisi 500 mg/kg	7.00±0.22[a]	3.42±0.06	3.58±0.22	0.24±0.02[a]	0.20±0.00
C.paradisi 1000 mg/kg	7.04±0.12[a]	3.78±0.18[a]	3.26±0.18	0.26±0.02[a]	0.12±0.02

TP: Total Protein, ALB: Albumin, GLO: Globulin, TB: Total Bilirubin, DB: Direct Bilirubin. Normal: Normal control, Untreated: Untreated diabetic control, *C.paradisi: Citrus paradisi* [a] $p < 0.05$ relative to diabetic control, [b] $p < 0.05$ relative to normal control. Values are expressed as Mean±SEM

Effects of *Citrus paradisi* on Histopathological Parameters (Liver)

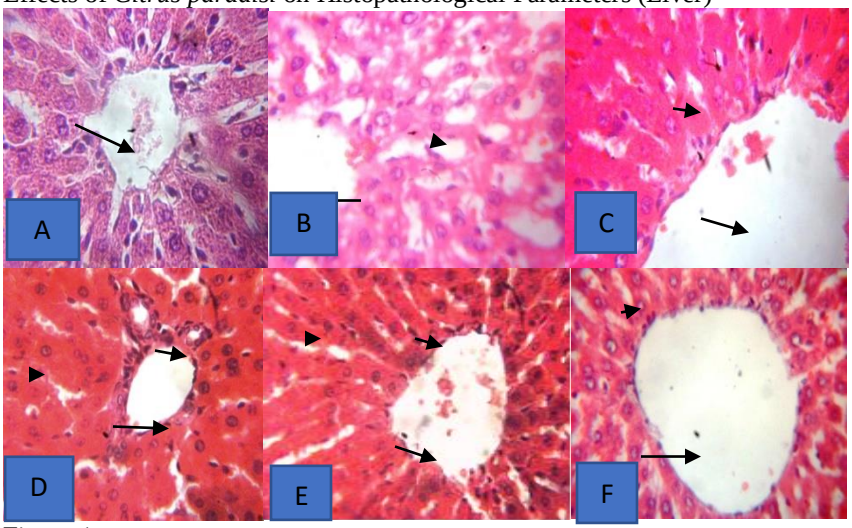

Figure 1: A. Normal control showing normal architecture with normal portal vein (arrow) B. Diabetic untreated liver showing congested portal vein (arrow) with visible fatty hydropic changes (arrow head). C. *Citrus paradisi* (250 mg/kg) reveals portal vein (long arrow) and radiating hepatocytes (arrow head). The nucleus does not appear distinct. D. *Citrus paradisi* (500 mg/kg) shows prominent hepatocytes with visible nucleus (arrow head). A prominent portal vein (long arrow) with few inflammatory cells is seen (short arrow) E. *Citrus paradisi* (1000 mg/kg) treated liver shows a distinct liver. The hepatocytes have pyknotic nuclei (arrow head) with inflammatory changes (short arrow). F. Glibenclamide (5 mg/kg) treated liver shows portal vein (long arrow) with mild hydropic changes (short arrow). Magnification (x 400).

Effects of *Citrus paradisi* on Histopathological Parameters (Kidney)

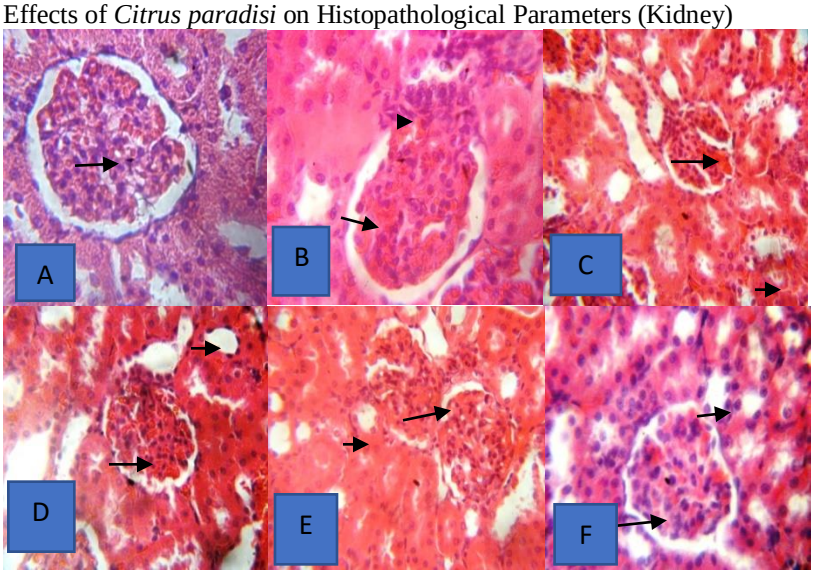

Figure 2: A. Normal control reveals normal architecture with renal corpuscles appearing as dense rounded structures (arrow) B. Diabetic untreated shows atrophied renal corpuscles (arrow) bounded by prominent inflammatory infiltrates (arrow head) C. *Citrus paradisi* (250 mg/kg) reveals atrophied glomerulus (long arrow) with mild focal tubular necrosis (short arrow) D. *Citrus paradsi* (500 mg/kg) treated group reveals normal cortical parenchyma and tubules (short arrow) with the renal corpuscles appearing as dense rounded enlarged structures (long arrow) E.*Citrus paradisi* (1000 mg/kg) treated group reveals normal cortical parenchyma and tubules (short arrow). The renal corpuscles appear as dense rounded enlarged structure with mild inflammatory changes (short arrow). F. Glibenclamide (5 mg/kg) treated group shows normal architecture with detailed cortical parenchyma (short arrow). The renal corpuscles appear as dense rounded enlarged structures (long arrow). Magnification (x 400)

Effects of *Citrus paradisi* on Histopathological Parameters (Pancreas)

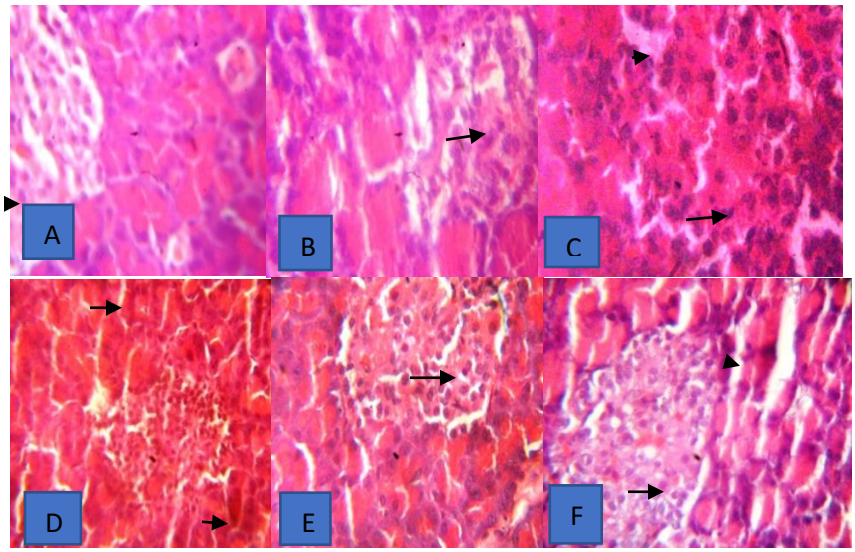

Figure 3: A. Normal control reveals normal architecture. Islet cells are seen embedded within acinar cells and surrounded by a fine capsule (arrow head) B Diabetic control shows acinar cells with islet cells having congested pyknotic nuclei and visible lymphocytic infiltrates (arrow head) C.*Citrus paradisi* (250 mg/kg) treated group reveals acinar cells with islet cells showing congestion and pyknotic nuclei (long arrow). There is some evidence of lymphocytic infiltration (short arrow) D. *Citrus paradisi* (500 mg/kg) treated group reveals a near normal architecture with the prominent secretory acini arranged in lobules E. *Citrus paradisi* (1000 mg/kg) treated group shows acinar cells with congested islet cells and pyknotic nuclei (long arrow). There are reduced lymphocytic nuclei (arrow head) F. Glibenclamide (5 mg/kg) treated group shows acinar cells arranged in lobules with prominent nuclei (long arrow). Some pyknotic nuclei are seen (short arrow). Magnification (x 400).

DISCUSSION

Diabetes and associated complications have continued to be a menace to societies in the first, second and third worlds hence the need to curtail its deleterious effects. The situation is particularly disturbing in third world countries like Nigeria where a significant percentage of the population cannot

afford orthodox antidiabetic medications hence the reliance on trado-medical care.

Herbal medicines are an important component in the management of diabetes mellitus especially in Africa explaining the need to evaluate their effects. *Citrus paradisi* (Grapefruit juice) which is obtained by expressing the fruit with the aid of a juice extractor is consumed as a part of the diet (Mohammed *et al.,* 2014). It is useful in the management of metabolic disorders, obesity and cardiovascular disease as a dietary intervention (Alam *et al.,* 2014). It has been advocated for use as a part of the diabetic diet in various circles. This advocacy is particularly of importance in the light of the fact that there exists an intricate link between diabetes. Obesity, dyslipidemia and hypertension. Chukwuma *et al* (2016) studied the effects of the white variety of *Citrus paradisi* on blood glucose and renal function of alloxan-induced diabetic rats. However, alloxan has been shown not to adequately mimic a model of type 2 diabetes mellitus when compared to streptozotocin hence the need for studies with streptozotocin. More so, the study didn't address haematological, hepatic, electrolyte and histopathological derangements associated with diabetes. Buchner *et al.,* (2014) studied the hepatoprotective and antioxidant effect of the purple species of organic and conventional grapefruit on rats fed with a high fat diet however this study did not make use of streptozotocin. Also, it did not make use of the white variety of the fruit which is more common in Nigeria.

The phytochemical screening of *Citrus paradisi* juice revealed the presence of carbohydrates, alkaloids, tannins, flavonoids, saponins and anthraquinones. Flavonoids found in *Citrus paradisi* such as naringin and narigenin have been cited to be partly responsible for the observed effects of *Citrus paradisi* and other citrus fruits on metabolic disorders, obesity, cardiovascular disorders and the non-alcoholic fatty liver disease (Alam *et al.,* 2014). In addition to these, an important class of compounds called furanocoumarins (e.g bergamottin) have been found in *Citrus paradisi*. Furanocoumarins and the flavonoids have been associated with the enzyme inhibitory properties of *Citrus paradisi* juice and other citrus species. They inhibit intestinal CYP3A4 and transporters such as p-gp and organic cation transporters thus affecting the levels of drugs metabolized or excreted through these pathways (Hung *et al.,* 2017). These interactions have been considered to contribute to the adverse effects of such drugs including calcium channel blocker and antiretroviral drugs. However, it is believed that these compounds could be exploited for use as pharmacokinetic enhancers thereby reducing the dosing requirements of these drugs particularly with the protease inhibitors (Kiani and Imam, 2007; Hanley *et al.,* 2011). The positive effects of the flavonoids on metabolic syndrome (Nzuza *et al.,* 2017) could be exploited to manage protease inhibitor- induced lipodystrophy. This might open new vistas in therapeutics (*Row et al.,* 2006).

The acute toxicity study performed on *Citrus paradisi* juice revealed no mortality at any of the doses used within the first 24 hours and during the two-week observation of the rats. The absence of mortality at doses of 5000 mg/kg shows that the juice is safe explaining why the juice is consumed freely as part of the diet of some individuals and is being promoted as a part of the healthy diet. The absence of mortality following the administration of *Citrus paradisi* to wistar rats agrees with the work of Owira and Ojewole (2009) who determined the median lethal dose of *Citrus paradisi* in wistar rats.

Weight loss is characteristic of diabetes mellitus due to the destruction of structural proteins and muscle wasting (Oyedemi *et al.,* 2009). The study revealed that the *Citrus paradisi* at various doses except 250 mg/kg ameliorated weight loss at the 2nd week of treatment ($p < 0.05$). This is in tandem with the work of Murunga *et al* (2016) which showed that naringin, a component of *Citrus paradisi* ameliorates weight loss in streptozotocin-induced type 1 diabetic rats. This study has therefore been able to show that *Citrus paradisi* is also useful in this regard in the type-2 diabetes model which we mimic in this case. The antioxidant effects of the flavonoids, tannins and furanocoumarins of *Citrus paradisi* prevent the formation of free radicals which are known to provoke the production of certain insulin counter-regulatory hormones hence opposing the anabolic actions of insulin might have contributed immensely to this effect (McPherson and McEneny, 2010).

Haematological alterations in diabetes mellitus have been reported. Diabetes-associated anemia is due to the non-enzymatic glycosylation of RBC membrane proteins which is directly linked with poor glycaemic control (Oyedemi *et al.,* 2011). Hyperglycemia and the oxidation of these proteins increase the synthesis of lipid peroxides which are responsible for the lysis of erythrocytes (Arun and Ramesh, 2002) hence, a reduction in erythrocyte survival time (Kolanjiappan *et al.,* 2002). Additionally, nephropathy as a complication of diabetes affects erythropoietin synthesis in the kidney (Ikemura *et al.,* 2012). When compared to the untreated diabetic group (hyperglycemic control), there was a significant difference ($p < 0.05$) in red blood cell count between the diabetic treated and the diabetic untreated groups. *Citrus paradisi* (250 mg/kg) was however an

exception. These observations lend credence to the work of Mahmoud (2013) who demonstrated that citrus bioflavonoids, naringin and hesperidin ameliorate diabetes-induced anemia in streptozotocin induced diabetic rats. The administration of *Citrus paradisi* juice (500 mg/kg and 1000 mg/kg) to streptozotocin induced diabetic rats showed significant elevations in erythrocyte count and other red blood cell parameters such as haemoglobin concentration, mean cell haemoglobin and mean cell haemoglobin concentration. These effects might be attributable to the presence of antioxidant principles present in the juice which could prevent the free radical induced damage of the erythrocytes. Naringin and its aglycone narigenin in citrus have an established antioxidant effect (Mahmoud and Hussein, 2016) which might play a role in this. Citrus bioflavonoids have been shown to scavenge for superoxide and hydroxyl radicals invitro (Cavia-Saiz *et al.,* 2010). Additionally, the nephroprotective effects of *Citrus paradisi* juice (Chukwuma *et al.,* 2016) is well documented. This nephroprotection could retain the kidneys ability to produce erythropoietin ultimately leading to erythropoiesis hence the improved RBC parameters seen in comparison to the RBC parameters of the diabetic untreated rats. It has been suggested that citrus bioflavonoids such as naringin and hesperidin could stimulate erythropoiesis by causing the production of erythropoietin (Ohlsson and Aher, 2012). This suggestion is corroborated by the evident increase in the MCH and MCHC levels in the *Citrus paradisi* treated diabetic rats relative to the diabetic untreated rats. These two parameters are indicators of the haemoglobin concentrations which show the oxygen carrying capacity of the blood (Mahmoud, 2013). The mean cell volume of the diabetic treated rats was not significantly different from the controls. This contrasts the work of Mahmoud (2013) who reported significant increases with naringin and hesperidin over the untreated group.

The leucocyte count of the diabetic untreated control was significantly ($p < 0.05$) higher than that of the treated groups showing that the extracts slowed the development of leukocytosis. This effect seen with *Citrus paradisi* corroborates the findings of Mahmoud (2013) who established the role of citrus bioflavonoids in correcting haematological abnormalities in diabetic rats hence, it can be said that the effects of the juice might in part be due to naringin. White blood cells have been shown to increase in response to a toxic environment and this is associated with insulin resistance, macrovascular and microvascular complications (Mahmoud, 2013). The decrease in the WBC count seen in the rats administered *Citrus paradisi,* might be due to the anti-inflammatory effect of certain phytochemical present in the extracts. Hesperidin and naringin, both flavonoids from citrus species have been cited to have anti-inflammatory effect which might play a role in correcting leukocytosis (Xu *et al.,* 2003; Mahmoud, 2013;). The differential white blood cell count did not show any significant difference between the untreated and treated groups. This observation contrasts with the work of Mahmoud (2013) who showed a significant difference in differential white blood cell counts amongst the treated and untreated diabetic groups.

A significant increase in the platelet count of the diabetic untreated rats in comparison to the *Citrus paradisi* treated rats was seen ($p < 0.05$) as the extracts ameliorated increases in platelet count. This might be attributed to the anti-inflammatory effect of some of the phytochemicals in the plant. Inflammation and infection are known to play a role in platelet hyperactivity (Osigwe *et al.,* 2017) and aggregation and is due to poor glycaemic control (Uko *et al.,* 2013).

From our study, in comparison to the diabetic untreated group, the *Citrus paradisi* treated groups showed significant reductions in ALP levels ($p < 0.05$). They also showed significant reductions in AST and ALT levels ($p < 0.05$). On the whole, the decrease in hepatic enzymes seen with *Citrus paradisi* extract could be attributed to the hepatoprotective and antioxidant effects of *Citrus paradisi* (Buchner *et al.,* 2014).

Treatment with *Citrus paradisi* ameliorated electrolyte derangements. The treated groups showed significant increments in bicarbonate levels and sodium levels but decreased potassium levels in comparison to diabetic controls. Bicarbonate levels have been shown to decrease due to acidosis (due to hyperosmolar non-ketotic hyperglycemia in type-2 diabetes). Sodium levels have been shown to decrease due to osmotic diuresis, with shift hyperkalemia being responsible for increased potassium levels (Liamis *et al.,* 2014). These were ameliorated by the administration of *Citrus paradisi* juice suggestive of an improved effect in the type-2 diabetic model. Murunga *et al* (2016) had shown the salutary effects of naringin on these electrolytes in a type-1 diabetic model. The insignificant difference between the chloride levels of the untreated and treated groups is however in contrast to the work of Murunga *et al* (2016) who reported decrements in the chloride levels of the streptozotocin-induced type-1 diabetic model while naringin treatment had no effect.

Serum liver transaminases were modulated by the administration of *Citrus paradisi* fruit juice. This effect could be partly attributed to the effects of the citrus bioflavonoids. Naringin supplementation has been shown to reduce plasma transaminases in nickel and cadmium-induced liver toxicity in rats (Renugadevi and Prabu, 2010; Pari and Amudha, 2011). It has also been shown to reduce fat deposits in rats fed with a high level of carbohydrates or fats (Alam *et al*., 2013). Numerous effects of naringin are believed to contribute to this including antioxidant and anti-inflammatory effects (Alam *et al*., 2013)

Decrements in serum proteins seen in the diabetic control rats when compared to the normal control are indicative of hyperglycaemia. The daily administration of *Citrus paradisi* offered a salutary effect. The antioxidant effect of *Citrus paradisi* may have contributed to this effect (Eleazu *et al.,* 2013). The citrus bioflavonoid, naringin has been shown to restore total protein and albumin levels in rats whose livers were damaged with dimethylnitrosamine prior to naringin administration (Lee *et al.,* 2004). Naringenin treatment provided a salutary effect on serum albumin and total protein levels and decreased the levels of malondialdehyde in the liver of rats administered dimethylnitrosamine (Lee *et al*., 2004).

Diabetic rats treated with *Citrus paradisi* (500 mg/kg and 1000 mg/kg) showed significantly reduced total bilirubin levels which is suggestive of hepatic improvement (Martin and Appel, 2010). The levels of direct bilirubin were unchanged.

The histopathology was quite informative. *Citrus paradisi* (500 mg/kg) juice extracts gave the best result from the liver photomicrograph as it was devoid of fatty changes though slight inflammatory changes were seen explaining the significant increase seen in their liver enzymes in comparison to the normal control. This is consistent with the work of Buchner *et al* (2014) who showed the role of purple *Citrus paradisi* of the Bordeaux variety in protecting the liver of rats fed with a high fat diet from developing hepatocellular damage and hepatic steatosis. It therefore follows that *Citrus paradisi* might have a role in preventing hepatic steatosis. These observations might be attributed to the presence of antioxidant and anti-inflammatory principles in *Citrus paradisi* such as naringin, narigenin, resveratol and vitamins C and E (Buchner *et al.,* 2014). Histopathological examination of kidneys of rats treated with *Citrus paradisi* (500 mg/kg) had an architecture better than that of the untreated rats as they were devoid of atrophy, necrosis or inflammation. The effects seen on the kidneys support a nephroprotective effect (Chukwuma *et al*., 2016). These observations might be attributed to the presence of antioxidant and anti-inflammatory principles present in the extracts. Fuji *et al* (2006) showed that the proanthrocyanidins had the strongest protective effect against high glucose mediated oxidative stress on the cultured kidney cells.

The pancreas of rats treated with 500 mg/kg *Citrus paradisi* juice revealed marked improvements over the diabetic untreated which had visible lymphocytic infiltrates, congestion and pyknotic cells. *Citrus paradisi* contains flavonoids such as naringin and its aglycone narigenin, hesperidin and anthrocyanidins (Alam *et al.,* 2016; Mahmoud and Hussein, 2016) which could help delay the progression of pancreatic cell damage by antioxidant effects (Eleazu *et al.,* 2013). Naringin and vitamin C co-supplementation modulated streptozotocin-induced diabetes in rats by improving insulin concentration and prevented oxidative stress (Alam *et al.,* 2016). It also improved insulin concentration and pancreatic architecture in mice (Jung *et al*., 2004)

Citrus paradisi, despite its medicinal benefits its notorious for drug interactions some of which pose danger to health. Understanding these interactions and managing them in patients taking them with orthodox drugs is essential. Furanocoumarins and flavonoids in the juice have been fingered to be responsible for the drug interactions via the inhibition of intestinal CYP3A4 and the permeability glycoprotein (Hung *et al.,* 2017). This has implications for drugs such as statins, calcium channel blockers, protease inhibitors, some meglitinides and thiazolidinediones (Kiani and Imam, 2007). Despite this, it is increasingly been suggested that these interactions could be exploited to therapeutic benefit (Kiani and Imam, 2007) to reduce the doses of drugs (hence reduced toxicity) and to boost other drugs such as protease inhibitors.

CONCLUSION

Carbohydrates, flavonoids, saponins, alkaloids, anthraquinones and tannins are some of the primary and secondary metabolites present in *Citrus paradisi* fruit juice. The juice is very safe as even at doses greater than 5000 mg/kg it elicited no toxicity. The administration of *Citrus paradisi* fruit juice modulated complications of diabetes in the streptozotocin-induced type-2 diabetic model. Flavonoids and furanocoumarins in the juice should be extensively studied and exploited therapeutically. In spite of these, extensive drug interaction studies need to be carried out to determine if there are harmful interactions when citrus juice is co-administered with other medications. This is because the furanocoumarins contained by *Citrus paradisi* are

known intestinal CYP 3 A4 and P-gp inhibitors. Current thinking suggests that these interactions should be exploited to therapeutic benefit. These considerations should be explored.

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