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EFFECT OF *HIBISCUS SABDARIFFA L. PIPER NIGRUM L.* AND *ZINGIBER OFFICINALE R.* EXTRACT ON SERUM ELECTROLYTES PROFILE AND CHANGES IN THE PANCREAS OF ALLOXAN INDUCED DIABETIC RAT.

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ABSTRACT

Diabetes Mellitus (DM) is the most common endocrine disease which is characterized by hyperglycaemia, altered metabolism of lipids, carbohydrates and protein with an increased risk of many complications such as liver and pancreas damage and altered serum electrolytes level. During the past few years many plants and spices have been used to manage DM. This work aimed to investigate the possible anti-diabetic effect of *Hibiscus sabdariffa L., Zingiber officinale roscoe* and *Piper nigrum L*. extract treatments in alloxan induced diabetic rats via studying pancreas, liver structure abnormalities and serum electrolytes level alteration. 30 male Wistar rats (130-180 g) were injected intraperitoneally and divided into five main groups each of 6 rats. The control group was injected with a single dose of saline solution (0.9% NaCl), diabetic group was injected with a dose of alloxan solution (170 mg/kg), high and low dose of 250 mg/kg and 500 mg/kg extract after induction of diabetes respectively and metformin treated group was injected at a daily dose 500 mg/kg metformin after induction of diabetes. Microscopic histopathology examination of pancreatic tissues showed decrease in islets of Langerhans size in the diabetic group, sinusoidal enlargement and increased fatty vacuoles in the liver tissues of the diabetic group. There were alterations in the serum electrolytes levels of the diabetic group. These abnormalities were healed after treatment of diabetic rats with extract which could have the ability to regenerate beta cells of islets of Langerhans. The present study could verify that the extract normalized the various serum electrolytes levels and histological abnormalities resulted due to diabetes metabolic disorders.

Word count: Diabetes mellitus, *hibiscus sabdariffa l, Piper nigrum l, zingiber officinale, electrolytes*

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycaemia linked with abnormalities in the absorption of glucose caused by a decrease in insulin secretion or insulin sensitivity, or both (Mardiah *et al*., 2014) It is identified by raised glucose concentration in the blood which can be due to failure in the formation of insulin or ineffective use of insulin in the body. High glucose concentration in the blood produces symptoms of frequent urination (polyuria), increased thirst (polydipsia), weight loss, blurring vision and increased hunger (Polyphagia) (Aba *et al*., 2014).

Untreated diabetes can cause complications. Acute complications include diabetic ketoacidosis and non ketotic hyperosmolar coma. Serious long term complications include heart disease, damage to the eyes, stroke, foot ulcer and kidney failure (Naresh, 2017). The pancreas is made up of two portions, the exocrine and endocrine. The endocrine makes and secretes insulin, glucagon, somatostatin and pancreatic polypeptide in the blood (Longnecker, 2014) .The secretion of insulin regulates the metabolism of carbohydrate and maintains glucose passage across the cell membrane. In diabetes mellitus, oxidative stress causes the breakdown of liver cells, which may lead to

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leakage of proteins and other major constituents of cells into the blood stream. Altered glucose homeostatic balance can lead to adverse effects in the whole body of an organism.

*Hibiscus sabdariffa L*L. is a plant commonly known as zobo leaf and usually used as beverage in Southeast Asia. *Hibiscus sabdariffa L*L. consists of polyphenolic acids, flavonoids and anthocyanin. Previous studies show that *Hibiscus sabdariffa L*L. possess anti oxidative characteristics and anti-atherosclerotic effects. Recent pharmacological studies also show that it can meaningfully reduce blood pressure and reduce raised blood glucose level in humans and experimental animals (Wang *et al*., 2011).

Zingiber officinale Roscoe belongs to the family Zingiberacea and genus Zingiber, locally called Ginger. Ginger is widely used as spice worldwide. It has been highly rated for its medicinal properties in Asia, India and Arabic herbal traditions. The major components of ginger are the volatile oils and phenol compound such as gingerols, Zingbrene and sogaols. Research work has been done to show that ginger has a good influence on glucose and lipid metabolism in the body (Maisa *et al*., 2016).

Piper nigrum L. (Black pepper) is a South Indian native spice, it is known for its medicinal and culinary properties. It has been widely evaluated for its anti-convulsant, anti-depressant, anti-oxidant, anti-mutagenic, hepatoprotective and most importantly its bio-enhancing property. A bio-enhancer is capable of enhancing bio-availability and bio-efficacy of a nutrient by promoting rapid absorption of the nutrient (Atal *et al*., 2016).

Alloxan-induced diabetes has been usually demonstrated as an experimental model of insulin dependent diabetes mellitus. Alloxan action in the pancreas is led by its rapid uptake by pancreatic beta cells as urea analogue of glucose that has been proposed to be one of the important features to determine alloxan diabetogenicity. Damage of the cells occurs as a result of the toxic action of alloxan (Nosiri *et al*., 2016).

Metformin has been in the market for more than 50 years and has been established as the first line agent of choice for the management of type 2 diabetes (Papanas and Maltezos 2009).

Diabetes is on the rise, it is no longer a disease of mainly rich nations, the prevalence of diabetes progressively increasing everywhere, mostly noticeable in the world's middle income countries (WHO, 2016). Universally, an estimated 422 million adults are suffering from diabetes in recent years, compared to 108 million in 1980 (Elkotby *et al*.,2018). Nigeria is the diabetic capital of Africa and the most populated in Africa with about 170 million people (Oputa and Chinenye 2015) There is a significant increase in the use of natural medicinal products for treatment of diabetes mellitus and its complications but more research still needs to be done to replace the use of drugs for treatment with natural products therapy - the reason for the study. Diabetes can lead to death if not properly managed.

Recently, much attention has been given to *Hibiscus sabdariffa L*L. extract only in developing new treatment for Diabetes mellitus. This study will give information on the effect of *Hibiscus sabdariffa L*L. ,and some spices (Zingiberofficinale Roscoe and *Piper nigrum L*) on diabetes mellitus and some complications of DM such as serum electrolytes profile and histological changes in the pancreas. It also gives information on natural products as a replacement therapy for management of DM. This study attempted to know the effect of *Piper nigrum L*. as a bio enhancer on the combined extract.

MATERIALS AND METHODS

Preparation of plant extract

Healthy and organically grown leaves of Hibiscus *Sabdariffa L*L.,*Piper nigrum L* and rhizomes of *Zingiber officinale roscoe* were purchased from Ilishan market, Ogun State, Southwest Nigeria. Dirts were removed from the plant materials by washing initially with clean water, then with distilled water as many times as possible. 1000g fresh plant leaves and rhizomes were air dried and ground to powder and dissolved in 1000ml of 100% methanol (MeOH) overnight. The mixtures were filtered and then the filtrates were transferred to clean vessels and evaporated to dryness.

Experimental animals

Healthy male wistar albino rats, 8-10 weeks weighing 130-180g were purchased from Animal Facility of Babcock University, Ilishan- Remo, Ogun State. Prior to the commencement of the experiment, the animals were housed in a well ventilated and well illuminated facility in the department of biochemistry and they were fed standard diet of rat pellets and clean water *ad libitum.* All experimental protocols in this study were undertaken in accordance with regulations as set out by the Babcock University Health Research Ethics Committee (BUHREC) and certificate of approval was obtained.

Experimental design

Alloxan was the chemical used to induce diabetes in the rats and metformin was the standard anti-diabetic drug used for treatment in this study as it is the mostly administered drug used as the first line medication for diabetes treatment .The rats were allowed to fast for 12 hours then diabetic induction of rats was done by intraperitoneal injection of freshly prepared alloxan solution at a dose of 170mg/kg body weight in 0.1M cold citrate buffer, PH 4.5. Blood was collected from the tail vein after 72 hours and glucose levels were determined using glucometer test kit (ACCU-CHECK, BG-CHECK). Animals were considered diabetic when blood glucose level values were above 200 mg/dl. After preliminary studies, doses of 250mg/kg and 500mg/kg were selected for treatment. Adult wistar albino rats were randomly divided into five groups of six rats each and assigned different treatments. Animals in group I (normal control) received normal saline orally. Group II animals (negative control) were induced with alloxan and received water only. In group III (CT 250), animals were induced with alloxan and administered extract dose of 250mg/kg. Group IV (CT 500) was the second treatment group and the animals were treated with 500mg/kg of the extracts after induction with alloxan. Lastly group V (Standard control) received the standard treatment drug (metformin) 500mg/kg after being induced with alloxan. The treatments were administered by oral gastric intubation once daily and they lasted for 14 days. The rats were weighed at the beginning of the

study, after induction of alloxan and at the end of treatment respectively.

Estimation of blood glucose

The blood glucose level was measured using the glucometer kit at the beginning of the study, after induction with alloxan, on the 3rd, 5th, and 7th day after induction and at the end of treatment respectively.

BLOOD COLLECTION

Experimental rats were sacrificed at the end of the experiment by spinal dislocation and blood samples were collected using ocular puncture. The blood samples were collected in heparinized bottles for serum concentration.

ESTIMATION OF SERUM ELECTROLYTES

Serum electrolytes analysis was carried out using a manual spectrophotometric method for enzymatic determination. It involved the use of minimal modification of reagents.

Histopathological examination of the pancreas and liver tissue

Pancreas and Liver were carefully excised, rinsed in ice-cold saline and stored in 10% formalin for tissue characterization. The paraffin embedded pancreas and liver were sectioned using a semi-automated microtome. The tissue sections were then mounted on glass slides using hot plate. Afterward, the tissue sections were deparaffinised by xylene and rehydrated by decreasing grade of ethanol dilution (100%, 90% and 70%). The sections were stained with haematoxylin and eosin (H&E) (Sheehan and Hrapchak, 1980) All stained slides were examined using light microscopy equipped with a digital camera under magnification.

Statistical analysis

Analysis of data was done using GraphPad Prism version 8.00 for windows (GraphPad Software, San Diego, California). Results were expressed as mean ± standard error of mean (S.E.M). The difference between the means were analyzed statistically using one-way analysis of variance (ANOVA; 95% confidence interval). The differences were considered statistically significant at p values < 0.05.

RESULTS

Effect of extracts on weight

Weight changes of control and experimental groups are shown in figure 1. From the graphical representation, other groups showed a significant decrease in weight changes as compared to the normal control.

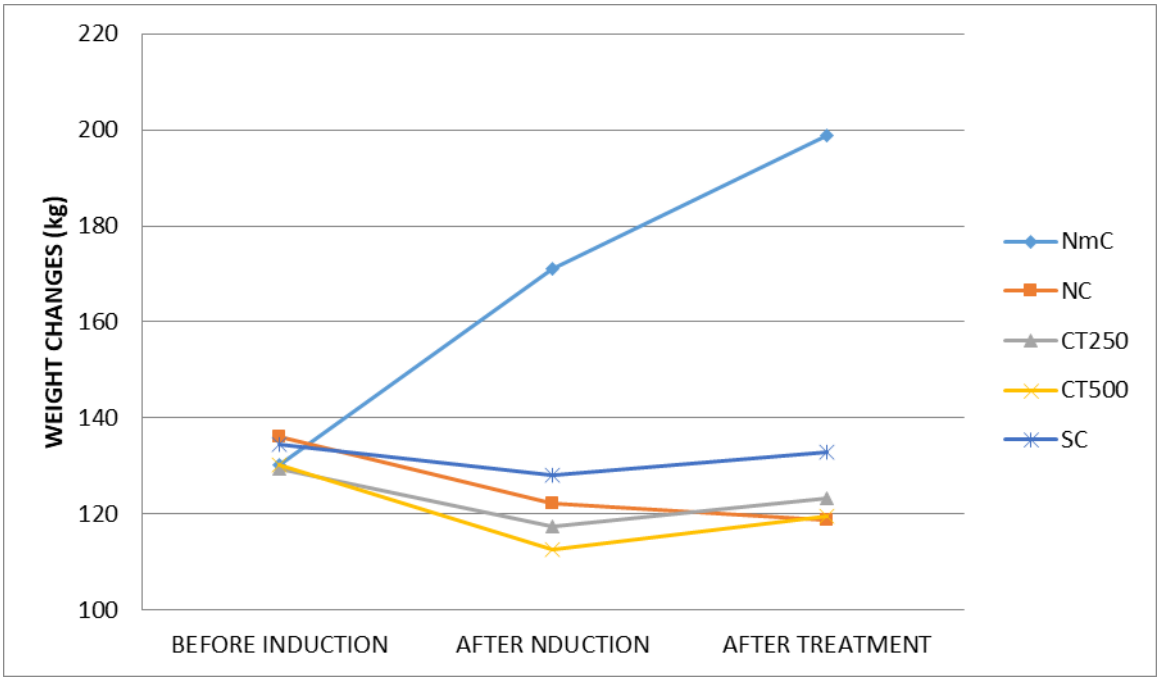

Fig 1: Weight gain levels of the normal control group, positive control group and chemo therapeutic groups.

NmC: Normal (normal glycemic) control group
NC: Negative (diabetic) control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: Standard control group treated with metformin

Effect of extracts on serum sodium levels

Serum sodium levels of controls and experimental groups are shown in Fig 2 below. The graph showed that the standard control group (metformin) had the highest serum sodium level as compared to the normal control and other groups, group 4 animals had the lowest serum sodium levels of all the groups

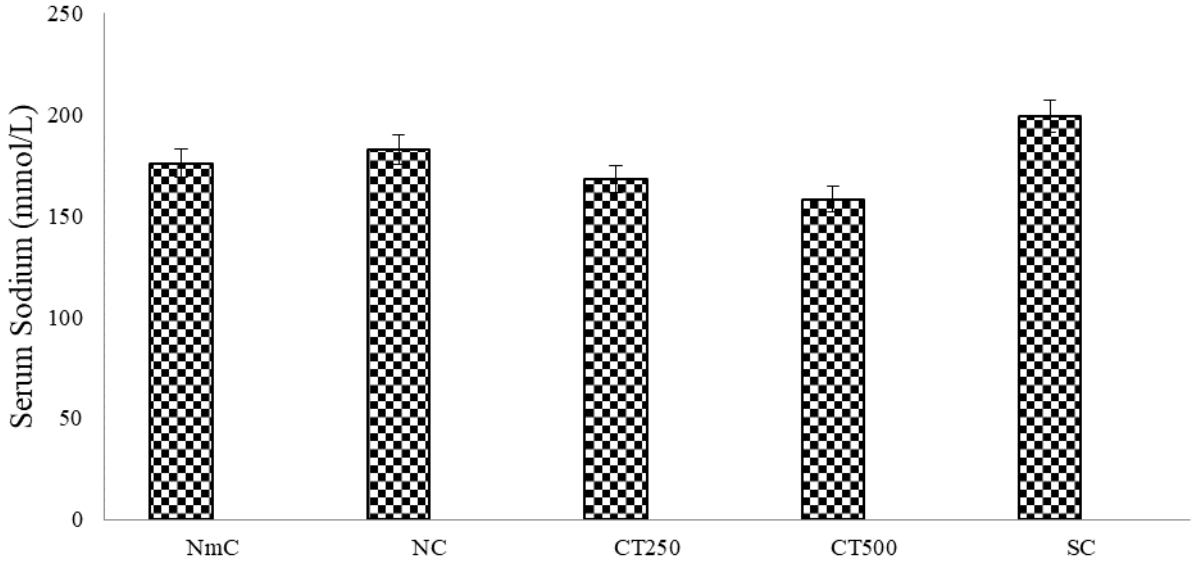

Figure 2: Effect of *Hibiscus Sabdariffa, Zingiber Officinale* and *Piper Nigrum* extract on serum sodium levels in alloxan-induced diabetic rats.

Results are presented as Mean ± SEM, *p<0.05 statistical significance

Effect of extracts on serum potassium levels

Serum potassium levels of normal control and experimental groups are shown in Fig 3 below. The graph shows that negative control (group II) had the highest serum potassium level while the group III had the lowest serum potassium levels of all the groups.

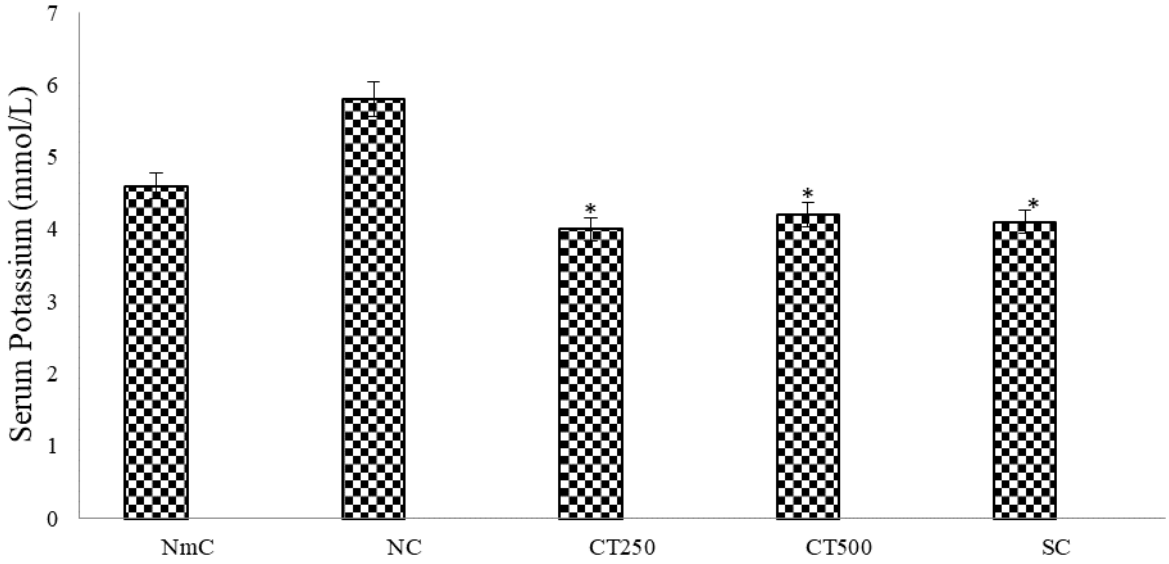

Figure 3: Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on serum potassium levels in alloxan-induced diabetic rats.

Effect of extracts on bicarbonate levels

Serum bicarbonate levels of normal control and experimental groups are shown in Fig 4 below. The graph shows that normal control (group I) had the highest serum bicarbonate level while the group II (negative control) had the lowest serum bicarbonate levels of all the groups.

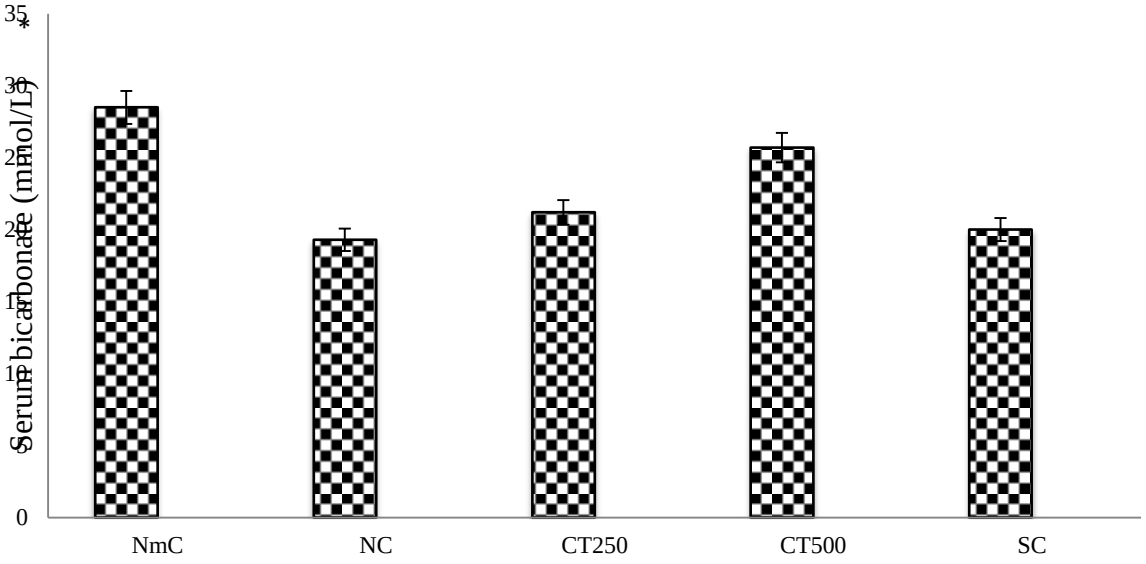

Figure 4: Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on serum bicarbonate levels in alloxan-induced diabetic rats.

Results are presented as Mean ± SEM, *p<0.05 statistical significance

Effect of extracts on blood glucose levels

Blood glucose levels of normal control and experimental groups are shown in Fig 5 below. The graph shows that negative control (group II) did not show a reduction in glucose level while the other groups had a drop in glucose levels following treatment with group 5 (standard control) showing the highest reduction in blood glucose.

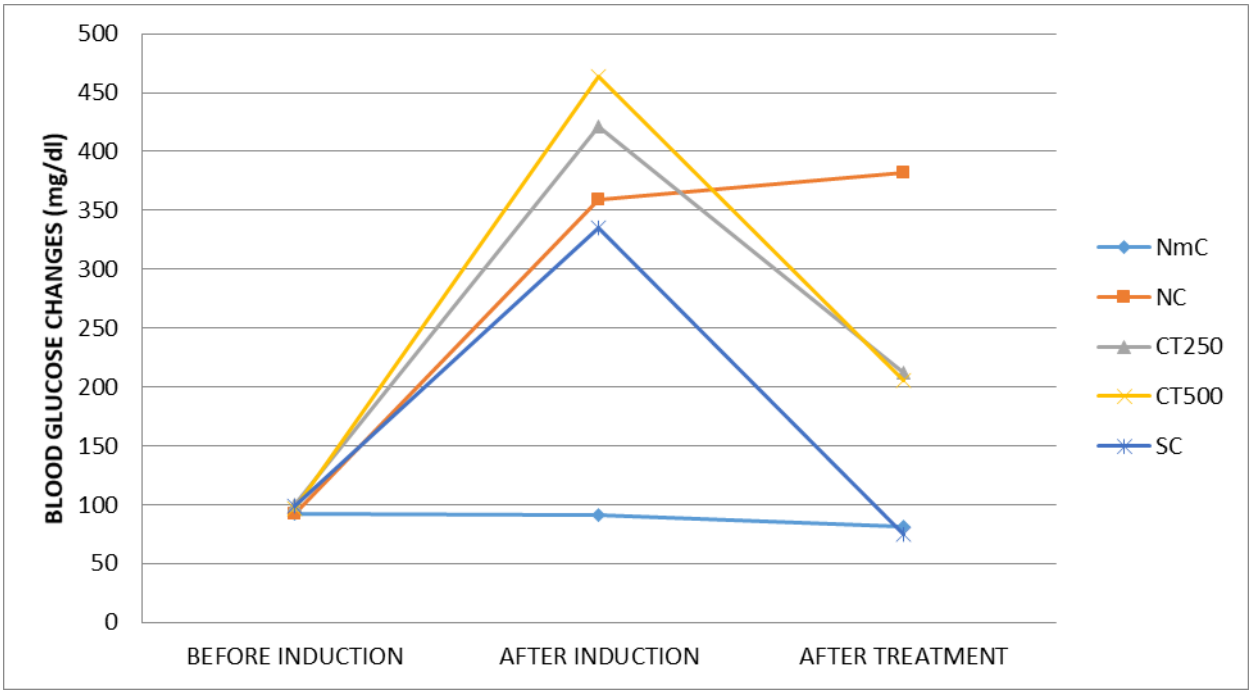

Figure 5: Blood glucose level of the normal control group, positive control group and chemo therapeutic groups during the oral glucose tolerance test.

NmC: Normal (normal glycemic) control group
NC: negative control group
CT250: chemotherapeutic diabetic group treated with 250mg/kg body weight of extract
CT500: chemotherapeutic diabetic group treated with 500mg/kg body weight of extract
SC: standard control (metformin) group

Effect of extracts on liver morphology

Photomicrographs of haematoxylin and eosin stained liver tissues of the control and treatment groups of rats used for the experiment. Magnification x 400

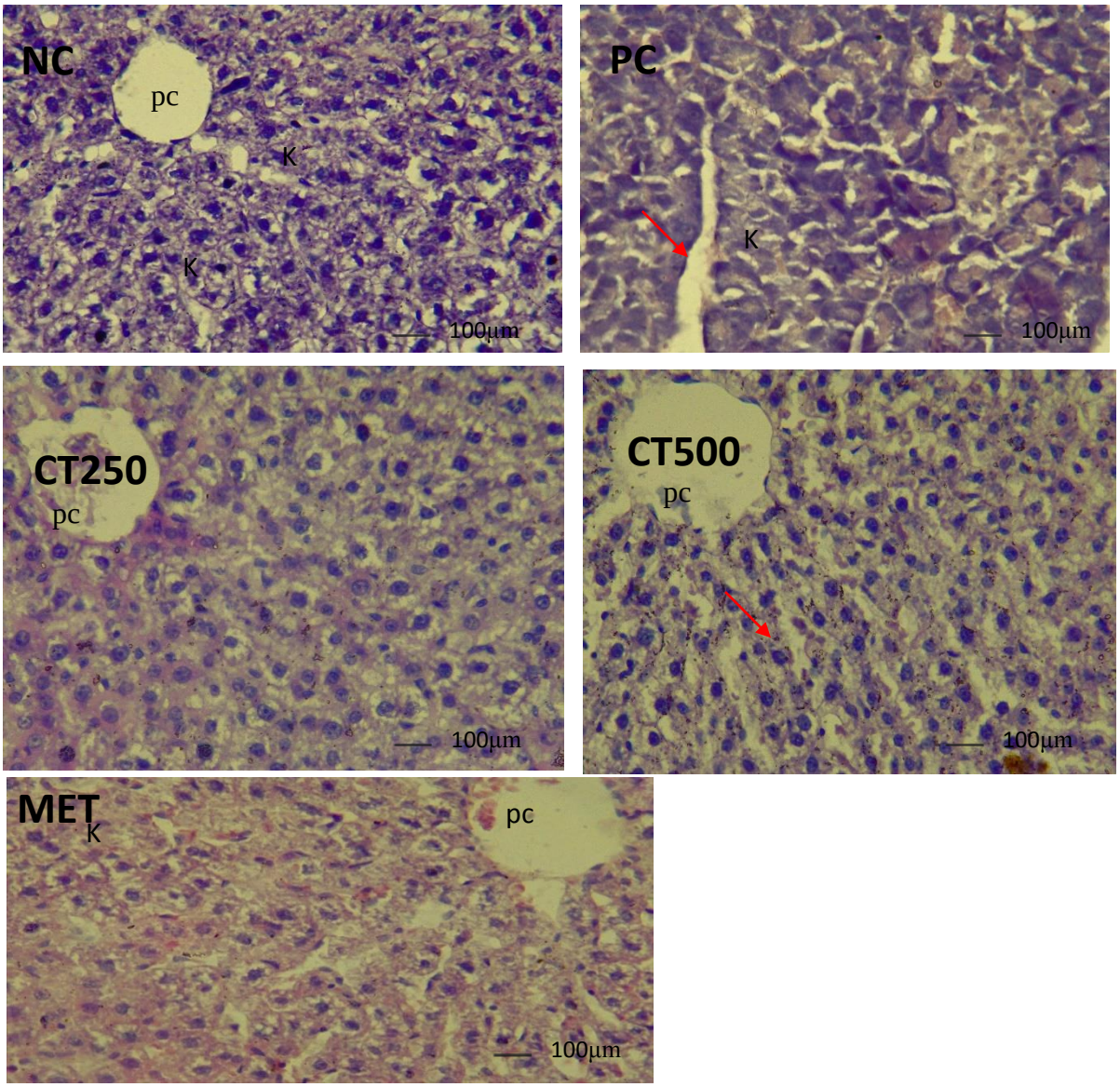

Plate 1: Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on the morphology of the liver in alloxan-induced diabetic rats.

Effects of extracts on pancreas morphology

Photomicrographs of haematoxylin and eosin stained pancreas tissues of the control and treatment groups of rats used for the experiment. Magnification x 400

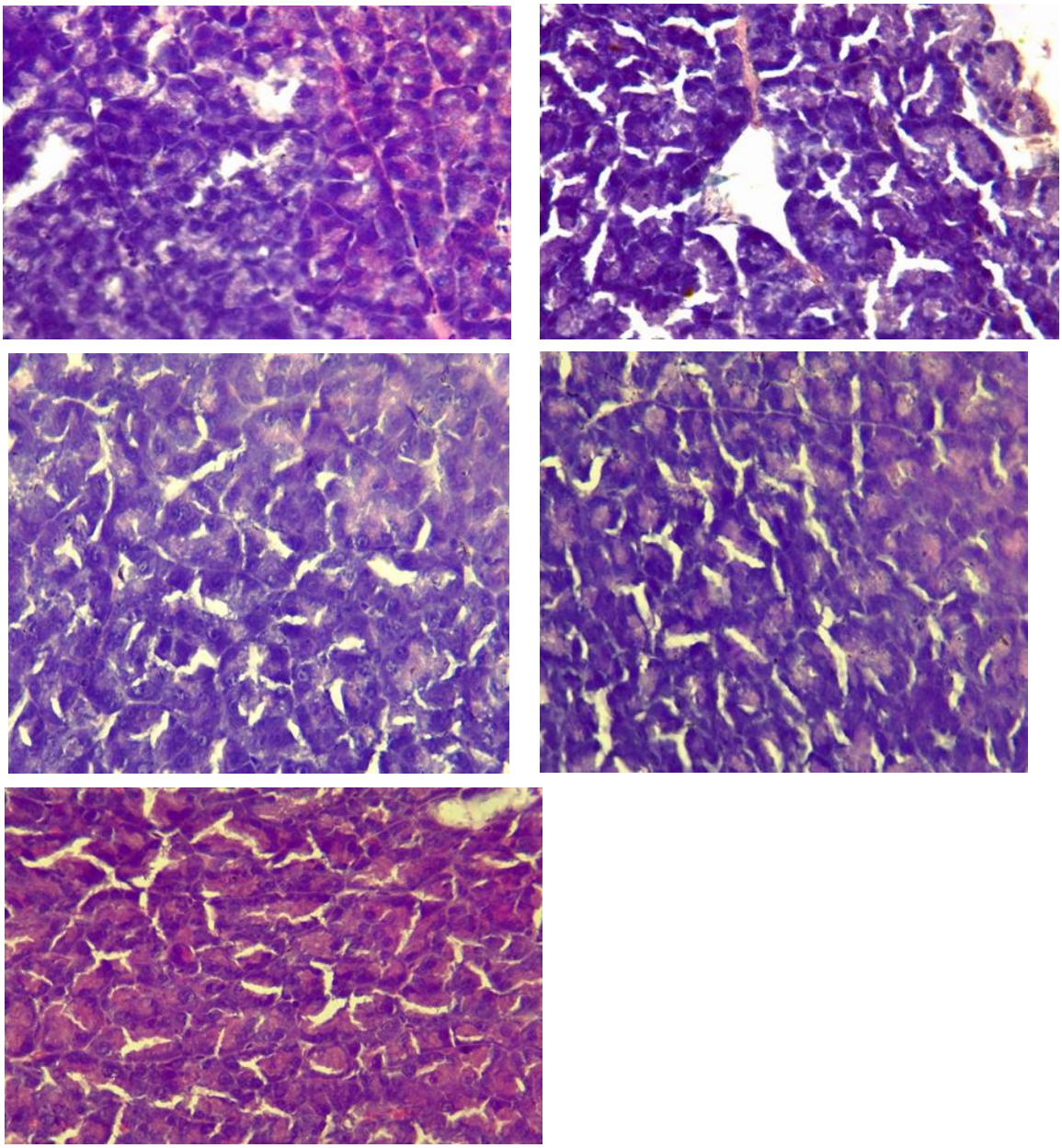

Plate 2: Effect of *Hibiscus Sabdariffa* and *Zingiber Officinale* and *Piper Nigrum* extract on the morphology of the pancreas in alloxan-induced diabetic rats.

DISCUSSION

This study investigated the effects of *Hibiscus Sabdariffa, Zingiber Officinale and Piper Nigrum* on diabetes and its complications. Upon induction of diabetes using alloxan, the blood glucose levels of the rats were significantly increased. Alloxan is a specific toxin that destroys the pancreatic beta cells thereby provoking primary deficiency in insulin without affecting other types of islets. This chemical leads to toxicity in pancreatic cells, which in turn reduces synthesis and release of insulin while concurrently affecting other organs and their metabolic activities, such as liver and serum electrolytes profile (Aba *et al*., 2014). Conditions such as hyperglycemia, polyuria, and weight loss are characteristics that may manifest in individuals suffering from diabetes. In this study, all these conditions were observed in alloxan induced rats. Oral administration of extract moderated the body weight of diabetic rats, caused a decrease in the blood glucose, moderated the sodium, potassium and bicarbonate electrolytes levels and repaired damaged liver and pancreatic tissues of diabetic rats.

A number of plants have been used traditionally to treat diabetes and some have proven to have hypoglycemic effects. *Hibiscus sabdariffa L* components include organic acids, flavonoids, polysaccharides which are suggested to be responsible for most of its pharmacologic activities. This study is in agreement with (Wang et *al*., 2014) who stated that *Hibiscus sabdariffa L*can meaningfully reduce raised blood glucose level in experimental animals. *Zingiber officinale roscoe* components include volatile oils and phenol compound such as gingerols, Zingbrene and sogaols. Ginger has a good influence on glucose metabolism in the body by reducing the blood glucose level, this study is correlating with (Maisa *et al*., 2016). Piper Nigrum has been widely

evaluated for its hepatoprotective and most importantly its bio-enhancing property (Atal *et al.,* 2016).

Electrolytes imbalance in diabetes is primarily a result of elevated blood glucose and their measurements is essential in DM. From the above results, there is a remarkable decrease in serum sodium, potassium levels and increase in bicarbonate levels in alloxan induced rats at 500 mg/kg as compared to 250 mg/kg and untreated rats. The extract suppressed generally this effect at 500 mg/kg, which is an indication of good management of DM. Pancreas histopathology data obtained from the study were consistent with (Elkotby *et al*., 2018) it shows significant histopathology effect ranging from mild to complete restoration in the pancrease of Alloxan diabetic rats treated with extract. Normal control rats were found to be stable while diabetic control group showed high level of cellular abnormalities. There is a significant difference in body weight of experimental animals recorded before and after induction, the decreased body weight in the diabetic rats is due to the deficiency of insulin, the storage fats and protein are broken down as an alternative source of energy thereby leading to reduction in weight (Atangwho *et al.,* 2012). Treatment of Alloxan induced rat with extract of 500 mg/kg/BW restores them to the normal state compared to 200 mg/kg/BW and might be useful for the management of DM.

Conflicts of interest: The authors declared no conflict of interest exist

Author's contributions:

All authors participated in research design. Author IFA, TEA and NCA conducted the research work, Author IFA and ARA wrote the manuscript. While Authors IJA, ABA and TEA revised the manuscript and the research design. All authors read and approved the final manuscript.

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