

Original Research Article

Effect of *Cucumis sativus* and *Prunus armeniaca* Juices On Blood Glucose, Insulin and Electrolytes of Alloxan Induced Diabetic Male Sprague Dawley Rats

Abstract

Aims: To investigate the effects of *Cucumis sativus* and *Prunus armeniaca* juices on blood glucose, insulin and electrolytes of alloxan-induced Diabetic male Sprague Dawley rats.

Study Design: An experimental study involving a total of eighty (80) male Sprague Dawley rats was used.

Place and Duration of Study: Department of Medical Laboratory Science, Rivers State University, Nigeria, between January 2021 and February 2022.

Methodology: 80 male Sprague Dawley rats were grouped into eight groups of ten rats each and housed in separate cages in an animal house. Group 1 was the positive control, group 2 negative control, group 3 high dose apricot, group 4 low dose apricot, group 5 high dose cucumber, group 6 low dose cucumber, group 7 a combination of apricot and cucumber juices, group 8 metformin. They were induced with 150 mg/kg alloxan intraperitoneally apart from group 1. The rats were treated for a period of 5 weeks and sacrificed. Blood glucose was estimated using the glucose oxidase method, electrolytes (Sodium, Potassium, Chloride) measured using serum electrolytes analyser, insulin was measured using Crystal Chem's Rat insulin ELISA method. Phytochemical analysis was done using Cleg Anthrone method for carbohydrate, Boham and Kocipaiabyazari test for flavonoid and Harbone method for alkaloids. Statistical analysis was done using GraphPad prism and results expressed as mean \pm SD ($P < 0.05$).

Results: The results showed that high dose cucumber significantly reduced ($P = 0.03$) blood glucose level compared to positive control; apricot and cucumber juices improved insulin level significantly ($P < 0.01$) compared to positive control; a combination of apricot and cucumber juices improved potassium level significantly ($P = 0.03$) compared to control. High Dose Cucumber also significantly reduced blood glucose level better than high dose apricot ($P < 0.01$).

Conclusion: Cucumber juice therefore improved diabetic condition in diabetic male Sprague Dawley rats and a combination of apricot and cucumber juices boosted potassium level in diabetic rats.

Keywords: *Apricot, Cucumber, Glucose, Insulin, Electrolytes, Diabetes, Rats*

1. INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder and a public health problem that occurs due to reduced activity of insulin and/or reduced insulin secretion. The disease which affects over 400 million people worldwide [1] is categorized into types one and two subtypes. Type 1 DM has autoimmune origin, affecting the pancreatic cells, thereby reducing or impairing insulin production. Type 2 DM is more common, occurring in 90-95% of diabetics as a result of impaired pancreatic beta cells that makes the individual unable to use insulin [2]

The use of oral hypoglycemic agents such as sulfonylurea-based drugs have been used by diabetics due to its ability to sufficiently elevate insulin secretion from β -cells to overcome peripheral insulin resistance and normalize blood glucose levels [3]. However, these synthetic drugs have certain adverse effects such as toxicity and weight gain. Hence, natural medicinal substances that can regulate blood glucose levels and induce insulin production by beta cells have become more attractive alternatives in the treatment and progression of this disease [4, 5]. Such plants include *Cucumis sativus* (cucumber) and *Prunus armeniaca* (apricot).

Cucumber is a vitamin, mineral and antioxidant-rich fruit that belongs to the Cucurbitaceae family [6]. It is rich in antioxidants and low in carbohydrates, thus touted as one of the most effective plants for

reducing blood sugar level in diabetes [7]. According to Minaiyan *et al.* [8], the plant has antihyperglycemic effects in some animal models due to its cucurbitacin content that regulates insulin release and hepatic glycogen. According to a study by Dixit *et al.* [7], cucumber peels ameliorate symptoms of diabetes mellitus in mice. Ethanolic extracts of this fruit have shown hypoglycemic effect on alloxan induced diabetic rats (AIDRs), reducing blood glucose by 67-87%. This is because it has α -glucosidase inhibitory activity [6]. Karthiyayini *et al.* [9] evaluated the antidiabetic activity of different doses of ethanol extract of *Cucumis sativus* on streptozocin-induced diabetic rats and found out that the fruit showed significant antidiabetic effects compared to standard drug [9]. Sharmin *et al.* [10] also studied the effect of cucumber ethanolic extracts on alloxan induced diabetic rats and showed that this edible plant significantly reduced the elevated blood glucose level in these rats [10]. Also, Mohammed *et al.* [11] studied the effect of aqueous fruit extract of *Cucumis sativus* on blood glucose levels in normal and streptozocin induced diabetic rats and found out that administration of *Cucumis sativus* juice possesses anti-diabetic activity against streptozocin induced rats [11]. The hypoglycaemic effect of this plant has also been traced to its ability to potentiate the insulin effect of plasma by stimulating insulin release from the remnant pancreatic β -cells or from the bound form [12]. There's also a possibility of extra-pancreatic action including stimulation of peripheral glucose utilization and enhancing glycolytic and glycogenic processes leading to decrease in glycogenolysis and gluconeogenesis [13]. The phytochemical content of this fruit also has hypoglycaemic activities and contributes to the antihyperglycemic effect of this fruit [10, 14].

Apricot (*Prunus armeniaca*) is a highly nutritious and healthy fruit, rich in essential nutrients such as vitamins, beta carotene, calcium and potassium. It is also rich in fibres, antioxidant constituents and hypoglycemic agents like anthocyanin. These compounds play a role in the ability of apricot to decrease glucose concentration in diabetes and thus considered a beneficial diet for diabetics [4]. Cui *et al.* [15] investigated the antidiabetic effect of apricot pulp soluble dietary fibre on diabetic rat models and discovered that apricot pulp relieved the symptoms of diabetic rats due to significant change in their blood glucose levels [15]. Rawi *et al.* [4] also studied the effect of dried apricot on sixty-seven T2DM patients aged 39-67 years' old who had no other interfering health problems. They found out that apricot significantly decreased the blood glucose of these patients Rawi *et al.* [4]. The antidiabetic effect of this fruit has been attributed to its rich fiber and antioxidant content [16].

Electrolyte disorders is a common, frequent development in diabetic patients. These disorders arise due to diabetic ketoacidosis or non-ketotic hyperglycemic hyperosmolar syndrome. As a result, the patients have depleted potassium, magnesium and phosphate levels. However, sodium levels may be high or low due to hyperglycemia-related mechanisms that changes serum sodium to opposite directions [17].

Increased local consumption of high-calorie western diets which increase post-prandial glycemic rates, contribute immensely to the development of metabolic diseases such as diabetes [18]. Diabetes spikes blood glucose level and resultantly affects electrolyte balance [19]. Synthetic hypoglycaemic agents abound but there is a continuous search for natural substances that could serve as alternative hypoglycaemic agents to toxic synthetic drugs [5]. Local fruits present as an exciting prospect due to their inherent beneficial phytochemical content [20]. The antihyperglycemic effects of various fruits have been studied. This study is therefore designed to investigate effects of *Cucumis sativus* and *Prunus armeniaca* juices on blood glucose, insulin and electrolytes of alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

2.1 Animal Preparation

The study was done using forty male Sprague Dawley rats acquired from the Animal house of the University of Port-Harcourt. The rats were housed, fed and acclimatized for two weeks before the experiment.

2.2 Study Design

This study is an experimental study. A total of eighty (80) male Sprague Dawley rats acquired from the Animal house of the University of Port-Harcourt were used for this study. The rats weighed an average of 150g. They were housed in separate cages and grouped into eight (8) groups, each group was made up of ten (10) rats:

Group 1: Ten rats in this group served as the negative control and so were not diabetes induced.

Group 2: Ten rats in this group served as the positive control. They were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate (Ighodaro *et al.*, 2017) and allowed food and water only ad libitum.

Group 3: Ten rats in this group were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate and treated with 0.8 ml/kg of apricot juice (high dose) (Francis *et al.*, 2017) for 35 days

Group 4: Ten rats in this group were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate and treated with 0.4 ml/kg of apricot juice (low dose) (Francis *et al.*, 2017) for 35 days

Group 5: Ten rats in this group were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate and treated with 0.8 ml/kg of cucumber juice (high dose) (Francis *et al.*, 2017) for 35 days

Group 6: Ten rats in this group were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate and treated with 0.4 ml/kg of cucumber juice (low dose) (Francis *et al.*, 2017) for 35 days

Group 7: Ten rats in this group were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate and treated with 0.8 ml/kg (Francis *et al.*, 2017) of a mixture of cucumber and apricot juices for 35 days

Group 8: Ten rats in this group were diabetes induced by intraperitoneal injection of 150 mg/kg alloxan monohydrate and treated with 70mg/kg of metformin (Zhang *et al.*, 2017) for 35 days

Overnight FBS of the rats was determined before administration of the extracts. Fruit juice administration was done for a period of 35 days. Animals were sacrificed and blood sample collected for laboratory analysis.

2.3 Fruit Collection

Two fruits were used in this study namely *Prunus armeniaca* and *Cucumis sativus*. The fruits were bought from a fruit market in Port-Harcourt and identified botanically by the Department of Plant Science and Biotechnology, University of Port-Harcourt. Juices were extracted from fruits and administered in known quantities to the rats.

2.4 Fruit Extraction

Fruits were washed and the bark (for apricot) peeled. The pulp of each fruit was then blended using an electric blender and the juice sieved into a water bottle and stored in a fridge at 2-8°C for 2 days. The seed of *Prunus armeniaca* was removed after peeling before blending but *Cucumis sativus* was blended with its seeds. Fresh juice was extracted every two days.

2.5 Induction of Diabetes

Animals were fasted overnight and their Fasting Blood Sugar determined using Acu-check glucose kit. Alloxan monohydrate was reconstituted using ice-cold injection water and administered intraperitoneally at a dose of 150mg/kg once to each of the experimental animals in groups 2 to 7. Animals with FBS greater than 135 mg/dl were considered hyperglycaemic.

Calculation

Alloxan Induction

$$\frac{\text{dose} \times \text{body weight}}{1000}$$

Dose = 150 mg/kg (Ighodaro *et al.*, 2017)

Average body weight of animal = 150g

$$\frac{150 \times 150}{1000} = 22.5 \text{g per rat}$$

For a group (5 rats) = 22.5 × 5 = 112.5 g

It was dissolved in 1 ml of normal saline

Fruit Dose 0.8 ml/kg for high dose and 0.4 ml/kg for low dose (Francis *et al.*, 2017)

$$\frac{0.8 \times 150}{1000} = 0.12 \text{ ml per rat for high dose}$$

Metformin administration

Dose = 70 mg/kg (Zhang *et al.*, 2017)

$$\frac{70 \times 150}{1000} = 10.5 \text{ mg per rat}$$

2.6 Phytochemical and Nutritional Studies

The fruit extracts were subjected to nutritional and phytochemical analysis. Carbohydrates, Sodium, Potassium, flavonoids, alkaloids were looked out for.

2.7 Method of Assay

2.7.1 Estimation of Fasting Blood Glucose Using Glucose Oxidase method by Trinder (1969).

Principle of Assay: Glucose oxidase catalyses the oxidation of glucose to produce hydrogen peroxide and gluconic acid. The hydrogen peroxide in the presence of peroxidase enzyme is broken down and the oxygen given off reacts with 4-aminophenazone and phenol to give a pink colour that is measured spectrophotometrically. The absorbance obtained is proportional to the concentration of glucose in the sample.

Procedure of Assay: The glucose reagents were brought to room temperature. Four tubes were set up and labelled test, standard, control and blank. 20µl each of Plasma sample, Glucose Standard and Control Sera were pipetted into the test, standard and control tubes respectively. pipetting done as shown in the table below. The content of the tubes were mixed and incubated at room temperature for 20 minutes. The spectrophotometer was set to the required wavelength (520nm) and zeroed using the blank. The absorbance of the test, standard and control were then read and calculated as shown below.

$$\frac{\text{Absorbance of test}}{\text{Absorbance of Standard}} \times \text{Concentration of Std.}$$

2.7.2 Estimation of Serum Electrolytes Using Clinical Serum Electrolyte Analyser

Principle: Clinical Serum Electrolyte Analyser uses Ion Selective electrode for electrolyte measurement based on the principle of potentiometry in which the voltage that develops between the inner and outer surfaces of an ISE membrane is measured (Khandpur, 2019).

Procedure: Clinical Serum Electrolyte Analyser KD100B was used for electrolytes estimation. The machine was switched on and maintained. Plasma sample free of hemolysis and lipaemia was used. Sample was mixed and aspirated through the sample probe and the result was displayed after few seconds. Results were recorded.

2.7.3 Estimation of Insulin Using Crystal Chem's Rat Insulin ELISA Method

Principle: It is based on a quantitative sandwich enzyme immunoassay technique. The microtiter plate has been pre-coated with monoclonal antibody specific for insulin. Samples are then added to the microtiter plate wells and if insulin is present, it will bind to the antibody pre-coated wells. A standardized preparation of horseradish peroxidase (HRP)-conjugated polyclonal antibody specific for insulin are added to each well to sandwich the insulin immobilized on the plate in order to quantitatively determine the amount of insulin present in the sample. The microtiter plate is incubated and wells washed to remove all unbound components. Substrate solutions are then added to each well. The enzyme (HRP) and substrate react over a short incubation period and only wells that contain insulin and enzyme-conjugated antibody exhibit a colour change. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and colour change measured spectrophotometrically at 450nm. A standard curve is plotted relating the intensity of the colour (O.D) to the concentration of standards. The insulin concentration in each sample is interpolated from the standard curve.

Procedure: 95 µl of diluent was added with 5 µl of sample and incubated overnight at 4°C. The plate was washed and 100 µl of conjugate solution was added and incubated for 1 hour at room temperature. The plate was washed again and 100µl of substrate solution added and incubated for 30 minutes. 100 µl of stop solution was then added and absorbance measured at 450nm.

2.8 Phytochemical Analysis

2.8.1 Estimation of Carbohydrates Using Cleg Anthrone Method (1956)

2.8.1.1 Principle

If carbohydrate is present in the form of free carbohydrate as poly- or monosaccharide or bound as in a glycoprotein or a glycolipid, the concentrated acid in the Anthrone reagent first hydrolyses it into component monosaccharide. Similarly, the concentrated acid then catalyzes the dehydration of the monosaccharides to form furfural (from pentoses) or hydroxyl furfural (from hexoses). The furfural or hydroxyl furfural formed condenses with two molecules of naphthol from the Anthrone reagent to form a blue-green complex. The complex can then be quantified by measuring the absorbance of 620 nm wavelength in a spectrophotometer or in a red filter colorimeter.

2.8.1.2 Procedure: 0.1g grinded sample was weighed onto a clean boiling test tube. 1ml distilled water was added to 1.3ml of 52% Perchloric Acid. The test tube was shaken to homogenize the mixture. The reaction was observed for 20 minutes for complete hydrolysis and was made up to 25.0ml with distilled water. The digest solution allowed to settle and 0.1ml was pipetted into a clean test tube. 0.9ml distilled water was added to make complete 1.0ml working test solution. 1.0ml distilled water was also pipetted into another clean test tube and labelled blank test solution. 0.1ml of 1% Glucose solution was prepared, pipetted into another clean test tube and this was labelled Standard solution. 1.0% Anthrone powder was weighed into a 100ml measuring cylinder and was made up to hundred ml mark with concentrated Sulphuric Acid. 5.0ml of this reagent was added to the test tubes respectively. The greenish blue colour formed was read at 630nm wave length, using blank test to zero the Spectrophotometer.

The absorbance of the sample and the Standard Glucose were read respectively and recorded.

$$\% \text{ Glucose as Carbohydrates} = \frac{\text{absorbance of sample}}{\text{absorbance of standard glucose}} \times 25$$

Where the weight of sample analyzed is 1.0g

2.8.2 Estimation of Flavonoids Using the Method of Boham and Kocipai (1974)

2.8.2.1 Procedure for Qualitative Analysis: 6 ml of 10% dilute ammonia solution was added to a portion of the aqueous literate of the plant extract, followed by addition of concentrated H₂SO₄. A yellow colouration observed in the extract indicated the presence of flavonoids

2.8.2.2 Procedure for Quantitative Analysis: 10g of the plant was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over water both and weighed to a constant weight

2.8.3 Estimation of Alkaloids Using the Method of Harborne (1973)

2.8.3.1 Procedure: 5g of sample was weighed into a 250ml beaker and 200ml of 10% acetic acid in ethanol was added, covered and allowed to stand for 4 hours. This was filtered and the extract was Concentrated in a water bath to one quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the preparation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed

2.8.4 Sodium and Potassium Estimation Using Atomic Absorption Spectrophotometry (AAS)

2.8.4.1 Principle: The ions in the sample solution are transformed to neutral atoms in an air/acetylene flame. Light from a hollow cathode or an electrodeless discharge (EDL)-lamp is passed through the flame. The light absorption of the atoms in the flame, which is proportional to the ion concentration in the sample, is measured by a detector following a monochromator set at the appropriate wavelength. The described principle holds for the measurement performed in the AAS-mode. In the AES-mode, the light emitted from the atoms exited in the flame is measured. Most commercial instruments can be run in both modes. Sodium may be measured more favourably in the emission mode.

2.8.4.2 Procedure: After a warm-up time of the instrument, the wavelength was set at 589.6nm for sodium and 766.5nm for potassium, and the slit width and the air/acetylene ratio also set. The flame was ignited and the reading of the instrument adjusted to zero by spraying the blank into the flame.

2.9 Statistical Analysis

GraphPad Prism 8.02 (California, USA) was the statistical software used for the analysis of bio-data obtained. Statistical tools used were mean, standard deviation and inferential statistics using one Way ANOVA, Post-hoc test was done using the Tukey's multiple comparison tests and P values less than 0.05 were considered statistically significant.

3. Results and Discussion

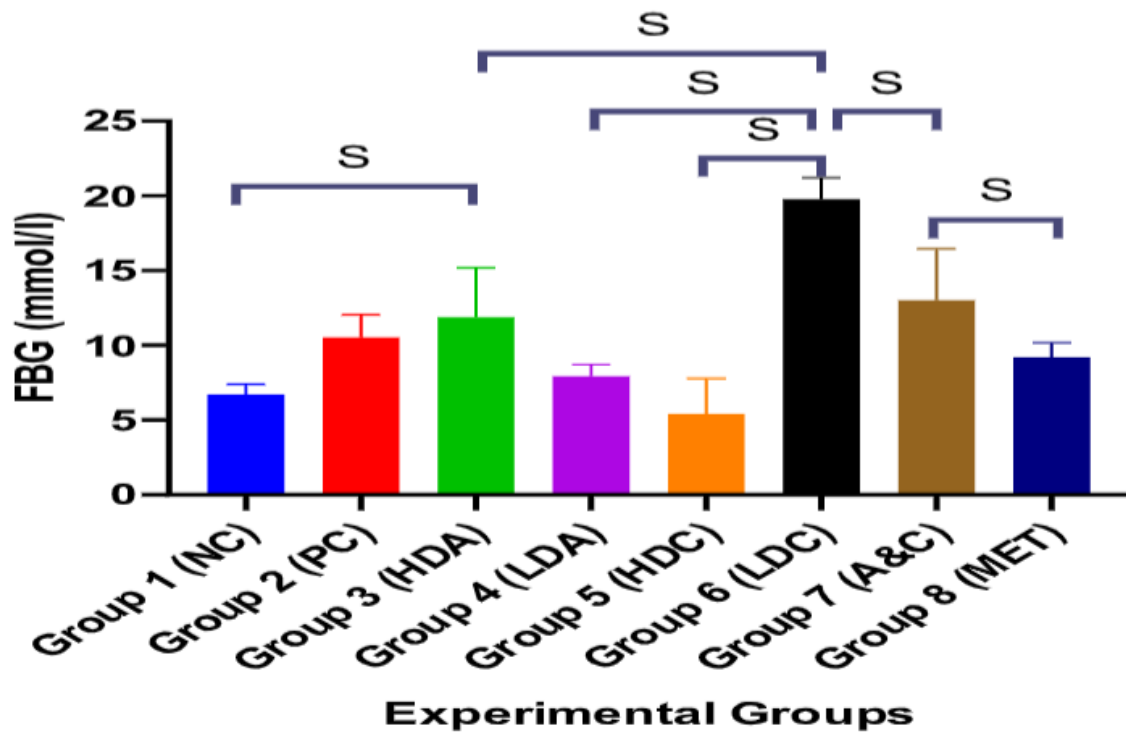


Figure 1 Significant Combinations for Fasting Blood Glucose (FBG)

Negative control (NC), positive control (PC), high dose apricot (HDA), low dose apricot (LDA), high dose cucumber (HDC), low dose cucumber (LDC), apricot and cucumber (A&C) and metformin (MET). S – Significant at $p < 0.05$.

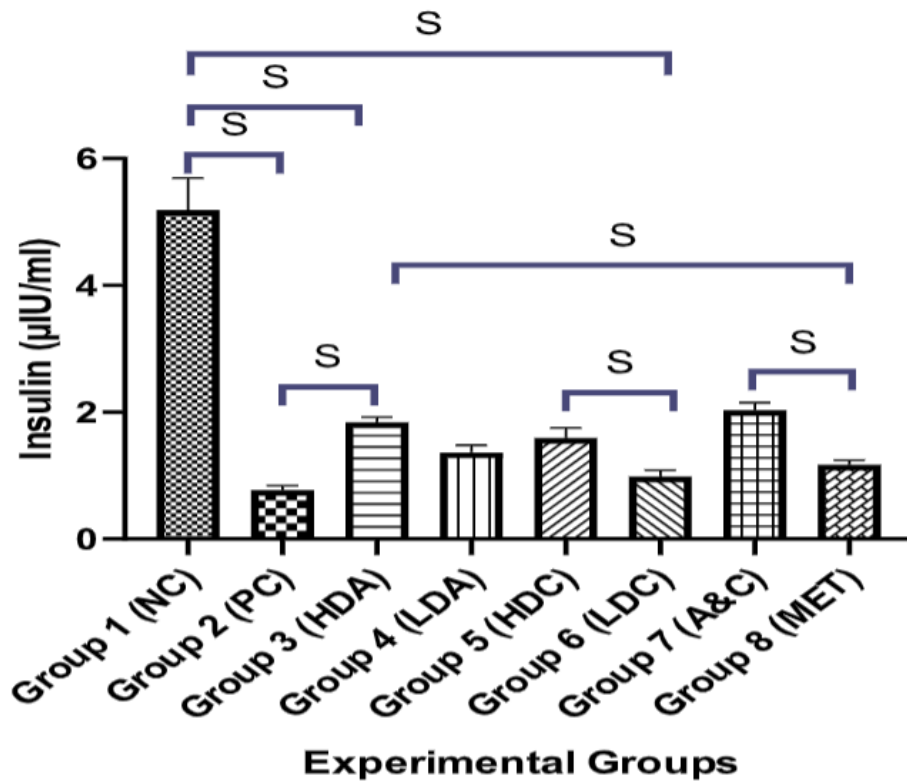


Figure 2 Significant Combinations for Insulin

Negative control (NC), positive control (PC), high dose apricot (HDA), low dose apricot (LDA), high dose cucumber (HDC), low dose cucumber (LDC), apricot and cucumber (A&C) and metformin (MET). S – Significant at $p < 0.05$.

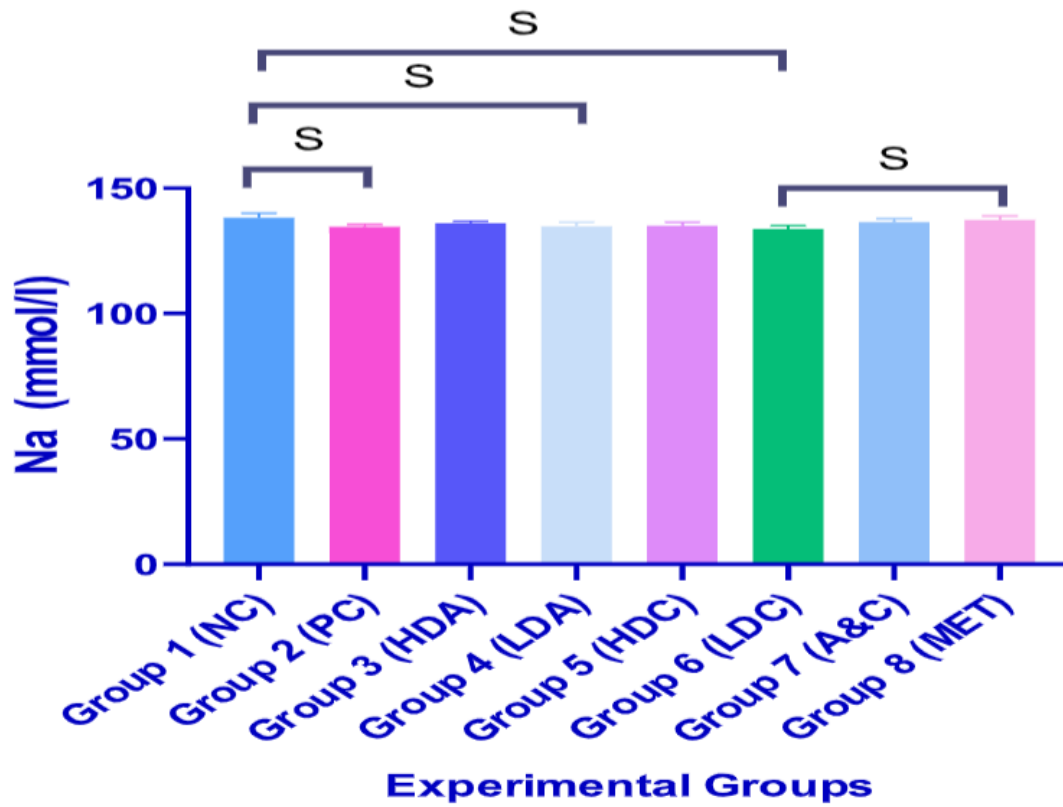


Figure 3 Significant Combinations for Sodium (Na)

Negative control (NC), positive control (PC), high dose apricot (HDA), low dose apricot (LDA), high dose cucumber (HDC), low dose cucumber (LDC), apricot and cucumber (A&C) and metformin (MET). S – Significant at $p < 0.05$.

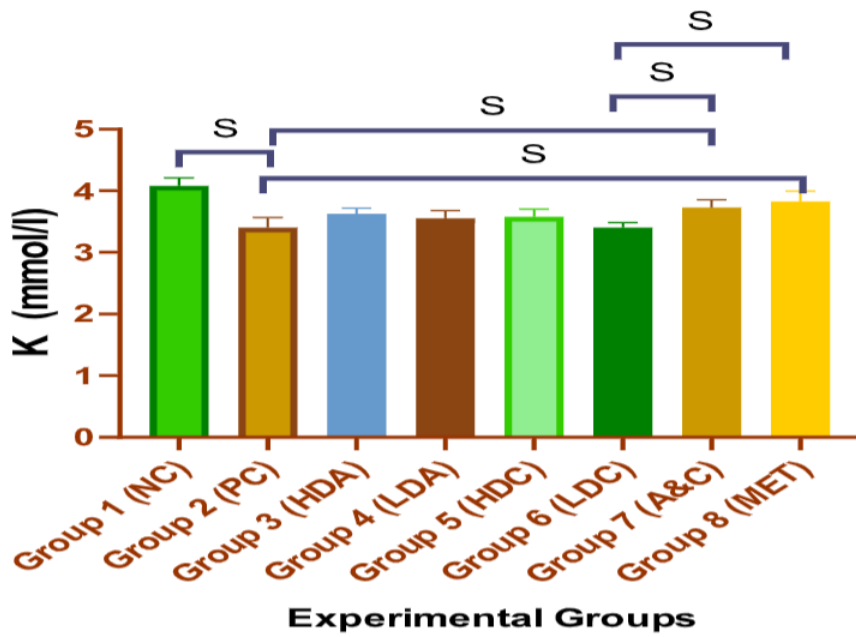


Figure 4 Significant Combinations for Potassium (K)

Negative control (NC), positive control (PC), high dose apricot (HDA), low dose apricot (LDA), high dose cucumber (HDC), low dose cucumber (LDC), apricot and cucumber (A&C) and metformin (MET). S – Significant at $p < 0.05$.

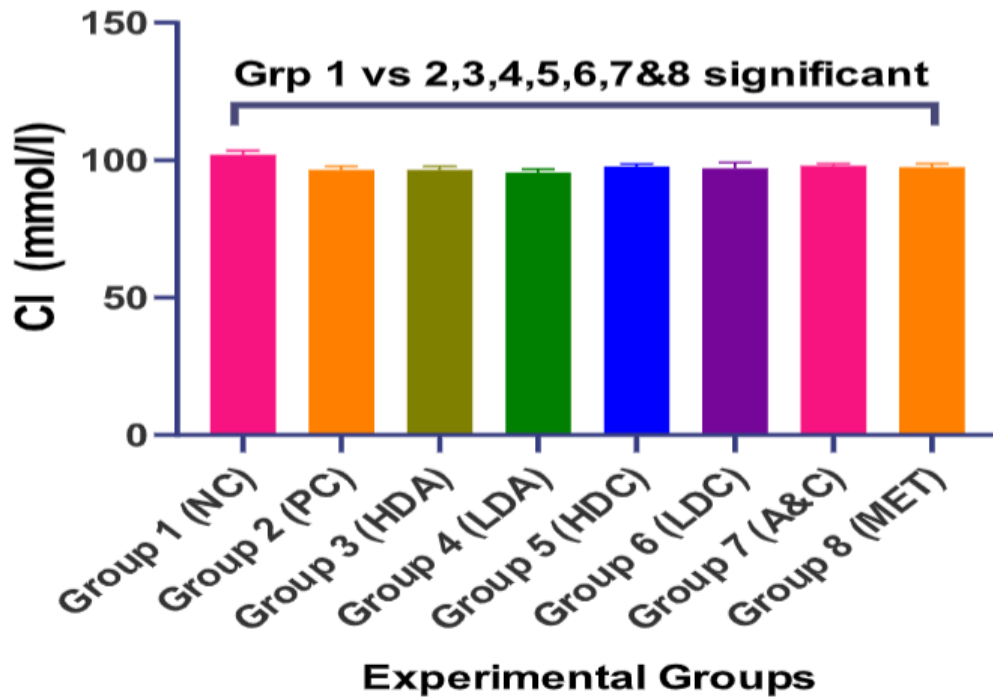


Figure 5 Significant Combinations for Chloride (Cl)

Negative control (NC), positive control (PC), high dose apricot (HDA), low dose apricot (LDA), high dose cucumber (HDC), low dose cucumber (LDC), apricot and cucumber (A&C) and metformin (MET). S – Significant at $p < 0.05$.

Discussion

The study indicated significantly lower blood glucose in diabetic rats treated with high dose cucumber (HDC) compared to positive control meaning that Cucumber juice possessed antihyperglycemic activity. This observation was in line with Karthiyayini *et al.* [9] who reported that *Curcuma sativus* showed significant antidiabetic activity compared to standard drugs. Sharmin *et al.* [10] also reported that Cucumber reduced elevated blood glucose levels in induced diabetic rats. According to Ojewole [14], this hypoglycemic effect is due to the ability of this plant to stimulate insulin release from remnant pancreatic B-cells and the hypoglycemic effect of its phytochemical content. Furthermore, Mukherjee *et al.* [6] also reported that ethanolic extracts of this plant has shown hypoglycemic effect in alloxan-induced diabetic rats due to its α -glucosidase inhibitory activity. There were increases in sodium level with high dose apricot and a combination of apricot juices treatment but this was not significant.

It was further observed in our study that blood glucose of diabetic rats treated with high dose cucumber juices was significantly lower than animals treated with high dose apricot juices and those treated with a combination of Apricot and Cucumber juices. This means that Cucumber juices alone had better antihyperglycemic activity than apricot juices and a combination of both apricot and Cucumber juices. This could be linked to the difference in the phytochemical content of both fruits. Apricot had a higher carbohydrates content but lower alkaloid and flavonoids content than Cucumber. Alkaloids and flavonoids exhibit antioxidant and anti-inflammatory activities which offer protection against oxidative stress mediated illnesses like diabetes [27]. Therefore, high dose cucumber juices have better hypoglycemic ability since they have higher amount of flavonoids, Alkaloids and lower carbohydrate content.

Our study also observed that although insulin level significantly reduces following alloxan induction, treatment with high and low doses of Apricot and Cucumber significantly increases insulin level in diabetic rats compared to positive control. Ojewole [14] and [28] both explain that the antioxidant content of both fruits can stop the oxidative stress mediated damage of B-cells and pave way for its repair. According to Gong *et al.* [29], removal of damage factors can drive B-cells to undergo redifferentiation and restore its function. Also, the cucurbitacin content of Cucumber helps regulate insulin release [6] and it can stimulate insulin release from remnant pancreatic B-cells [14].

Finally, our study showed that treatment of diabetic rats with a combination of Apricot and Cucumber juices significantly increases potassium level compared to positive control. Hyperglycemia in diabetic state causes induced osmotic diuresis in the internal environment resulting in a dilutional effect on the concentration of electrolytes, resulting in depletion of body fluid and electrolytes [30, 31]. However, a combination of Apricot and Cucumber juices improved potassium levels significantly since they are rich in electrolytes such as Sodium and potassium (evident from the phytochemical analysis). Campbell *et al.* [32] also reported that apricot is rich in potassium. Treatment with high dose apricot also increased potassium level but this was not also significant. High dose cucumber and a combination of Cucumber and apricot juices also increased potassium levels in diabetic rats but these were not also significant.

4. CONCLUSION

Our study indicated that Cucumber juice improved diabetic condition in diabetic male Sprague Dawley rats and a combination of apricot and cucumber juices boosted potassium level in diabetic rats.

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