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EFFECT OF GREEN TEA AND BLACK COFFEE ON KIDNEY PROFILE OF WISTAR RATS

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ABSTRACT

This research investigated the effects of green tea (*Camellia sinensis)* and black coffee (*Coffee arabica)* on the kidney function of Wistar rats. A total number of 20 experimental animals were used. These rats were separated into 4 groups and treated with green tea and black coffee extracts for a period of 3 weeks after being induced with hyperglycemia and hyperlipidemia. Sucrose and margarine were used to induce hyperlipidemia and hyperglycemia on the rats alongside normal rate feeds with the exception of the positive control that was not induced. Negative control was induced without treatment. Green tea group was treated with 2ml of green tea extracts, black coffee group was treated with 2ml of black coffee extracts. On spectrophotometric analysis, the results from this study showed with 95% confidence level that green tea and black coffee decreased the levels of electrolytes, urea and creatinine. Upon treatment with green tea on the third week of treatment, urea level had a decrease of 6.20±0.20 mmol/L when compared to negative control 6.60±0.30 mmol/L. On treatment with black coffee on the third week of treatment, urea level had a decrease of 6.50±0.25 mmol/L when compared to the negative control 6.60±0.30 mmol/L. Furthermore, upon treatment with green tea on the third week of treatment, creatinine level had a decrease of 127.00±1.00 µmol/l when compared to negative control 130.00±1.00 µmol/l. On treatment with black coffee on the third week of treatment, creatinine level had a decrease of 128.00±1.00 µmol/l when compared to the negative control 130.00±1.00 µmol/l. Histopathological analysis of the kidney after 3 weeks of treatment with green tea and black coffee shows a histologically normal kidney with intact glomeruli, patent bowman's capsule and intact renal tubules. This shows that green tea and black tea did not show any adverse effect on the histology of the kidney. This research shows that drinking of green tea and black coffee in moderation and not for prolonged period of time improves general wellbeing.

KEYWORDS: Black coffee, Electrolyte, Creatinine, Green tea, Kidney, Urea.

INTRODUCTION

Green tea is more than just a hydrating beverage. The green tea plant contains a range of healthy compounds that make it into the final drink. Tea is rich in polyphenols, which are natural compounds that have health benefits, such as reducing inflammation and helping to fight cancer (Kris, 2020; Sabu et al 2010). It is made from unoxidized leaves and is one of the least processed types of tea. For this reason, it contains the most antioxidants and beneficial polyphenols (Megan, 2021; Nagma and Hasan 2007; McKay and Blumberg 2002). Green tea is a type of tea that is made from Camellia sinensis leaves and buds that have not undergone the same withering and oxidation process used to make oolong teas and black teas (Khan and Mukhtar, 2013; Shahin et al 2008). The flavonoids in green tea have been proven to fight inflammation in the body. The component epigallocatechin gallate in green tea has anti-inflammatory effects and protects the cells. There are also antiviral, antibacterial, and antifungal properties in green tea which also work against inflammation (Lacey, 2021; Williams et al 2004).

Green tea is also known to have a calming effect when it is ingested. While it does provide caffeine, green tea also contains an amino acid called L-theanine, which produces a calming effect. The combination of caffeine and L-theanine has also been shown to optimize brain function to enhance working memory, cognitive performance, and elevate mood. Green tea’s ability to counter oxidative stress also makes it a potent protector against neurodegenerative diseases, including Alzheimer’s and Parkinson’s (Cynthia, 2020; Sumit et al 2011; Harold 1992). Green tea has other benefits which includes improvement of weight loss, increase satiety, lower risk of heart disease, reduce the risk of Esophageal

Cancer, reduce cholesterol, slow tooth decay and lower blood pressure (Ciara, 2021; Hazel 1999).

The chemical composition of green tea is a complex consisting of proteins (15-20% dry weight) whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such asteanine or 5-N-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, sucrose; lipids as linoleic and a-linolenic acids; sterols asstigmasterol; vitamins (B, C, E); xanthine bases such as caffeine and theophylline; pigments as chlorophyll and carotenoids; volatile compounds such as aldehydes, alcohols, esters, lactones, hydrocarbons, etc.; minerals and trace elements (5% dry weight) such as Ca, Mg, Cr, Mn, Fe, Cu, Zn, Mo, Se, Na, P, Co, Sr, Ni, K, F, and Al. Polyphenols constitute the most interesting group of green tea leaf components, and in consequence, green tea can be considered an important dietary source of poly-phenols particularly flavonoids (Cabrera *et al.,* 2006; Joseph 2000).

Green tea is rich in polyphenolic compounds, with catechins as its major component. Studies have shown that catechins possess diverse pharmacological properties that include anti-oxidative, anti-inflammatory, anti-carcinogenic, anti-arteriosclerotic and anti-bacterial effects (Koo and Cho, 2004). Green tea can be used to treat aches and pains, indigestion, depression. It is also used as a detoxifier, and as an energizer, and in general, to prolong life.

Black coffee is simply coffee that is normally brewed without the addition of additives such as sugar, milk, cream, or added flavors. Coffee is a drink made from coffee beans, which are the roasted fruit of the Coffea arabica bush. Coffee has a stimulating effect in humans, primarily due to its caffeine content (Cappelletti *et al,* 2015; Poole et al 2017; Bunker and McWilliams 1979).

Coffee contains a psychoactive chemical known as caffeine, an adenosine receptor antagonist that is known for its stimulant effects (Cappelletti, 2015). Coffee also contains the monoamine oxidase inhibitors β-carboline and harmane, which may contribute to its psychoactivity (Herraiz, 2006). Caffeine is rapidly and completely absorbed in humans, with 99 percent being absorbed within 45 minutes of ingestion (Liguori *et al.,* 1997). Polyphenols in coffee have been shown to affect free radicals in vitro, (Bakalar and Nicholas, 2006) but there is no evidence that this effect occurs in humans. Polyphenol levels vary depending on how beans are roasted as well as for how long (Williams *et al.,* 2004).

Black Coffee is rich in caffeine and acid, which means that excess consumption can lead to acidity in the stomach. It is likely to cause cramps and abdominal spasms. Too much Black Coffee release high levels of stress hormones in the body, which only leads to anxiety and stress. It can be easy to feel jittery after consuming too much caffeine. Too much coffee in the human system also makes it difficult for the body to absorb minerals from daily diet, such as iron, calcium and zinc (Poole et al 2017).

The kidneys play a pivotal role in the regulation of electrolyte and acid-base balance. Electrolytes play a vital role in maintaining homeostasis within the body. Electrolyte is any of the various ions (such as sodium or chloride) that regulate the electric charge on cells and the flow of water across their membranes. Electrolytes are important because they are what cells (especially those of the nerve, heart, and muscle) use to maintain voltages across their cell membranes and to carry electrical impulses (nerve impulses, muscle contractions) across themselves and to other cells (Tim 2018).

Some common Electrolytes include sodium, potassium, chloride, bicarbonate.

Creatinine is an important indicator of kidney health because it is an easily measured byproduct of muscle metabolism that is excreted unchanged by the kidneys. Creatinine is a waste product produced by muscles from the breakdown of a compound called creatine. Creatinine is removed from the body by the kidneys, which filter almost all of it from the blood and release it into the urine (Blann 2014; Agha 2003).

Urea is a waste product of many living organisms, and is the major organic component of human urine. This is because it is at the end of chain of reactions which break down the amino acids that make up proteins (Walter, 2005).

SIGNIFICANCE OF THE STUDY

Different research has been carried out on the effects of commonly ingested plant extracts, but specific research on the effects of green tea and black coffee in the regulation of electrolytes, urea and creatinine is novel.
The results obtained from this study will show the effects of aqueous extract of Green Tea (*Camellia sinensis)* and Black Coffee (*Coffee arabica)* on the Electrolytes, Urea and Creatinine level and Histology of Kidney of Wister Albino Rats.

AIM OF STUDY

The aim of the study is to determine the effects of Green Tea (*Camellia sinensis)* and Black Coffee *(Coffee arabica)* on Electrolytes, Urea, Creatinine and Histology of the kidney of Wistar Albino Rats.

MATERIALS AND METHOD

COLLECTION AND IDENTIFICATION OF TREATMENT MATERIALS

Processed and Packaged Green Tea and Black Coffee were purchased at a supermarket located at Choba junction, Obio-Akpor Local Government Area, Rivers

State. They were taken to the Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Choba for proper identification.

Experimental Animals

Twenty (20) albino rats (Five weeks old) with an average weight of 100g were purchased from the Department of Biochemistry animal house, University of Port Harcourt, Choba. They were acclimatized for a period of one week and fed growers mash. They were all kept in plastic cages with iron nettings in the animal house and given tap water at pleasure using plastic water bottles.

Grouping of experimental animals

The 20 albino rats were grouped in 4 cages with each cage containing 5 rats.

Group 1

This first group was labeled negative control, it was induced with hyperlipidemia and hyperglycemia but was not treated with the Green Tea nor Black Coffee extract.

Group 2

This group was labeled Green Tea, animals in this group were treated with green tea extract after being induced with hyperlipidemia and hyperglycemia.

Group 3

This group was labeled Black Coffee, the 5 animals in this group were treated with black coffee after being induced with a hyperlipidemic and hyperglycemic diet.

Group 4

This fourth group was the normal control, it served as the positive control. Rats in this group were not induced with hyperlipidemia and hyperglycemia or treated with any extract.

FORMULATION OF FEED

Hyperlipidemia was induced using high dose of margarine and egg yolks while Hyperglycemia was induced using granulated sugar (sucrose). The table below shows the percentage composition of the feed formulation to achieve hyperlipidemia and hyperglycemia.

Table 2.1: Feed Formulation.

INGREDIENT	% COMPOSITION	COMPOSITION BY WEIGHT (g)
Normal feed (Growers mash)	70%	2100
Margarine	15%	450
Egg yolk	10%	300
Granulated sugar	5%	150

Inducement of Hyperlipidemia and Hyperglycemia

Apart from the normal control group (Group 4, which were fed with Growers mash) every other groups were fed with the above formulated feed for up to a month, and a pilot study was conducted to ensure the disease condition has been induced on the animal.

Test for Hyperglycemia

Fasting Blood Sugar test

Procedure: Blood sample was collected from the animal before it was fed for the day. The blood sample was dropped on a glucose test strip and the strip is inserted into the Glucometer. The reading from the Glucometer was read. Results read from the Glucometer were divided by 18 and recorded.

Treatment of Kidney with Green Tea and Black Coffee

Preparation of Extracts (Green Tea and Black Coffee)

100ml of already processed Green Tea and Black Coffee was brewed in a kettle with 500ml of water for 30 minutes, then they were allowed to cool before administration to the animals.

Administration of extracts

According to previously outlined groups, the rats in the cages were treated according to the labeling on the cage, except the negative and positive control groups that were left untreated.

Group 1 served as the High Fat Control Group (negative control) and was not treated.

Group 2 was treated with Green Tea extracts with a dose of 2ml/kg body weight 3 times a day.

Group 3 was treated with a 2ml dose of Black Coffee per kg of body weight 3 times daily.

Group 4 served as the Normal control (positive control) and was neither induced nor treated with the extracts.

Administration of the extract was carried out orally by the use of syringes in order to determine the dose. The animals received their dose 2 times daily for a period of 3 weeks. The rats in each group were sacrificed at the end of each week. In each sacrifice blood samples were collected via cardiac puncturing. The kidneys were also dissected, collected and put in a labeled sample bottles for histopathological analysis.

Mode of sacrifice

A desiccator was made ready by pouring a potent amount of chloroform unto the piece of cotton wool placed at the base, the animal was then placed horizontally over the cotton wool and the lid of the desiccator was covered tightly. The rats remained in the desiccator for about a minute to inhale the chloroform (the effect of the chloroform leads to dizziness and suffocation which causes the rats to pass out). With a gloved hand, a sharp surgical blade was used to slice the body cavity of the animal open and a syringe was used to suck out its blood. Each blood sucked out was poured into a lithium heparin

bottle that has been labelled accordingly. The necessary organs were carefully removed and placed in a non-vacuum blood tube and were taken to the lab for analysis. This process was done for each sacrificial day.

TEST FOR ELECTROLYTES (SODIUM, POTASSIUM AND BICARBONATE)

Sodium (Trinder (1951) and Maruna (1958) method) mmol/L.

Principle: The present method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly as the concentration of sodium in the test sample.

Procedure: In this procedure, 3 test tubes were labeled as standard, blank, and test. Then 1.0ml of the reagent was pipette into all test tubes. 0.01ml of the samples we're added into each appropriate tube. The reagent is thoroughly mixed with the sample and incubated for 5mins at 25°C. It was read in the spectrophotometer at an absorbance of 630nm and readings recorded.

Potassium (Tietz (1995) method) unit mmol/L

Principle: The amount of potassium is determined by sodium tetraphenyl boron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentrating in the sample.

Procedure: In this procedure, 3 test tubes are labeled as standard, blank and test. 1ml of the reagent is add to all the test tubes, and 10ul of the sample into the appropriate test tubes. It was mixed thoroughly and allowed to stand for 3 minutes at 25°C. The spectrophotometer was zeroed using the blank at 500nm, then the absorbance was read and recorded.

Bicarbonate ($HC0_3$) (Pauss et al (1990) Back Titration Method) unit mmol/L

Principle: Serum HCO_3 is reacted with excess standard HCL. The remaining HCL is back titrates with standard NaoH using phenol red as indicator.

Procedure: In a 50ml conical flask, CO_2-free d/w 250ul, 200ul sample, 0.01N HCL 1ml were added and mixed very well, then 3 drops of phenol red was added to the mixture. The flask was whirled to release the $C0_2$. The resulting solution was titrated with 0.01 NaoH until the initial light yellow colour fades to a light purple at the end –point. The remaining NaoH that does not take part in the reaction was read. The reading obtained was divided by two; this will give the concentration of HCO_3 in the sample unit mmol/L.

Chloride: (Levinson S.S (1976) Method) unit mmol/L

Principle: The quantitative displacement of thiocyanate by chloride from mercuric thiocyanate any subsequent formation of a red ferric thiocyanate complexes is measured calorimetrically.

Procedure: 3 test tubes were first labeled as standard, test and blank. Then 1.0ml of the reagent was pipetted into all the tubes. 10 ul of the samples were added into the appropriate tubes and mixed thoroughly. This mixture was incubated for 5mins at 25'c.

It was read with a spectrophotometer at an absorbance of 480nm.

Urea determination

Urea levels were determined by enzymatic colorimetric endpoint method. The principle of this method is that urea is hydrolysed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in the presence of sodium nitroprusside as coupling agent to yield a blue cromophore. The intensity of the colour formed is proportional to the concentration of urea in the sample (Tietz, 1995).

The reagent kit contained reagent 1: (urease >500U/ml), stabilizers. Reagent 2/; (buffered chromogen), phosphate buffer (20mmol/l pH 6.9), EDTA (2 mmol/l), sodium salicylate (60 mmol/l), sodium nitroprusside (3.4mmo/l). Reagent 3: Alkaline hypochlorite, sodium hypochlorite (10 mmol/l), NaOH (150mmol/l), urea standard, urea (8.3 mmol/l). The working reagent was prepared by mixing 1ml of reagent 1 with 24 ml of reagent 2.

Exactly 1.00ml of the working reagent was mixed with 10µl of the sample. The standard tube contained 1.00ml of the working reagent and 10µl of the standard. The blank tube had 1.00ml of working reagent. The mixture was incubated for 5 minutes at 37°C and absorbance of sample read against the reagent blank at 600nm with Spectronic-20 spectrophotometer.

Calculations

Concentration of Sample $= \underline{\Delta A_{sample}}$ x $C_{standard}$ = mg/dL urea x 0.1665 = mmol/l $\Delta A_{standard}$

Normal values: 2.5 – 6.6mmol/l.

Creatinine determination

Creatinine levels were determined by colorimetric method (with deproteinization).

The principle of this method is that creatinine in alkaline solution reacts with picrate to form a coloured complex (Henry, 1974).

The Reagent kit contained solution 1: Standard (177umol/l), solution 2: Picric acid (35 mmol/l), solution 3: Sodium hydroxide (1.6 mol/l), TA 651 Trichloroacetic acid (TCA) (1.2mol/l). The Working reagent was prepared by mixing 10ml of solution 2 and 10ml of solution 3.

The sample was first deproteinized by mixing 1.0ml of Trichloroacetic acid (TCA) and 1.0ml of sample. The

mixture was vigorously stirred with a glass rod to evenly disperse the precipitate. The mixture was then centrifuged at 2500 rpm for 10 minutes, the supernatant was then separated and used for the assay as listed below.

Exactly 1.00ml of the working reagent was mixed with 1.00ml of the supernatant. The standard tube contained 1.00ml of the working reagent, 0.5ml of TCA and 0.5 ml of solution 1. The blank tube had 1.00ml of working reagent, 0.5ml of TCA and 0.5ml of distilled water. The mixture was let to stand for 20 minutes at 25 °C and the absorbance of the sample and standard were read against the blank at 520nm with Spectronic -20 spectrophotometer.

Calculations.

$$\frac{\Delta A_{sample}}{\Delta A_{standard}} \times 177 = \mu mol/l$$

Normal values : 44 – 80 µmol/l

KIDNEY HISTOLOGY

Tissue processing method

- The tissues were fixed using 10% formalin for 7days. The process is known as fixation. The method used for this fixation is paraffin wax methods.
- The tissues were dehydrated using ascending grades of alcohol 30% 50% 70% (3-12hr)90%(3-12hrs) Absolute1(3-12hrs) Absolute2(3- 12hrs) Absolute 3(3-12hrs).
- The tissues were cleared (de-alcoholisation) using xylene (15- 30mins). This is done to remove all alcohol content from the tissue sample.
- The tissues were impregnated using paraffin wax for2-3hrs. This process is also known as infiltration. It helps to remove clearing agent from the tissue
- The tissues were embattled with molten paraffin wax in a plastic ice-tray, the tray was immediately transfer to cold water to harden the wax. This process enables the tissue to section properly.
- The block were attached a holder
- The tissues were cut using base-sledge microtome
- The section tissues were floated in warm-water and pick-up with a slide.
- The slides were incubated to remove the wax end fix the tissues on the slide
- The slides were stain with Haematoxylin and Eosin.
- The slides were mounted using Canada balsam or DPX
- The slides were examined using x 400 objective lens

Statistical analysis

Data analysis was performed using the Statistical package for the Social Sciences software (SPSS, version 11.0). Data is displayed in mean + SD. The statistical method of one way analysis of variance (ANOVA) was used to compare the mean values obtained among different groups. Differences were considered significant whenever the p-value is P<0.05.

RESULTS

Table 1: Weight Measurement.

Group	Before Inducement (g)	After Inducement (g)	After one week of Treatment (g)	After two weeks of Treatment (g)	After three weeks of Treatment (g)
Negative Control (Induced, no treatment)	87.8	156.5	212.5	236.0	265.0
Green Tea (Induced & treated)	107.0	181.2	224.0	195.5	197.0
Black Coffee (Induced & treated)	106.0	186.6	208.0	216.3	214.0
Positive Control (Not induced or treated)	117.2	151.0	169.4	171.3	188.5

PILOT STUDY FOR HYPERGLYCEMIA

Table 2: Blood Glucose level analysis

This analysis were done in triplicates and expressed in their mean values.

Group	Before Inducement (mmol/L)	After Inducement (mmol/L)	After one week of Treatment (mmol/L)	After two weeks of Treatment (mmol/L)	After three weeks of Treatment (mmol/L)
Negative Control (Induced, no treatment)	5.2 ± 0.20[a]	7.1 ± 0.16[a]	8.3 ± 0.10[a]	6.0 ± 0.10[a]	4.7 ± 0.10[a]
Green Tea (Induced & treated)	5.7 ± 0.11[a]	6.8 ± 0.10[a]	5.1 ± 0.10[a]	5.1 ± 0.12[a]	4.3 ± 0.10[a]
Black Coffee (Induced & treated)	6.1 ± 0.10[b]	7.2 ± 0.12[b]	5.4 ± 0.20[b]	4.8 ± 0.10[a]	4.1 ± 0.20[b]
Positive Control (Not induced or treated)	5.8 ± 0.10[a]	5.8 ± 0.10[a]	5.6 ± 0.10[a]	5.4 ± 0.10[a]	5.2 ± 0.15[a]

Results are expressed as Mean ± Standard Deviation, n=3, values with different superscript are statistically different at ($P < 0.05$). Values with (a) superscript are significantly different from Positive control at $p < 0.05$ and values with (b) superscript are significantly different from Negative control at $p < 0.05$

TEST FOR ELECTROLYTES, UREA AND CREATININE

These analysis were done in triplicates and the results expressed in their mean values and standard deviation.

Table 3: Effect of Green Tea and Black Coffee on Electrolytes, Urea And Creatinine Levels After First Week Of Treatment.

Sample	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)	Urea (mmol/L)	Creatinine (µmol/l)
Negative control	5.10±0.26[a]	155.00±13.74[a]	61.00±4.00	22.00±2.00	4.80±1.20[a]	99.00±5.56[a]
Green Tea	3.30±0.10[b]	123.00±14.73[b]	52.00±1.00[b]	24.00±2.00	4.70±0.10	95.00±2.00[ab]
Black Coffee	3.50±0.43[b]	131.00±6.08	53.00±1.73[b]	26.00±2.64	4.60±0.17	92.00±2.00[ab]
Positive Control	3.20±0.10[b]	114.66±12.74[b]	56.00±1.73	24.00±2.64	5.80±0.90[b]	117.00±2.00[b]

Results are expressed as Mean ± Standard Deviation, n=3, values with different superscript are statistically different at ($P < 0.05$). Values with (a) superscript are significantly different from Positive control at $p < 0.05$ and values with (b) superscript are significantly different from Negative control at $p < 0.05$.

Table 4: Effect of Green Tea and Black Coffee on Electrolytes, Urea And Creatinine Levels After Second Week Of Treatment.

Sample	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)	Urea (mmol/L)	Creatinine (µmol/l)
Negative Control	6.00±0.26[a]	165.00±2.64[a]	65.00±1.00[a]	24.00±2.00	7.00±0.26[a]	140.00±2.00[a]
Green Tea	4.50±0.10[b]	135.00±2.64[b]	50.00±1.73[b]	28.00±2.00	5.30±0.10[b]	110.00±6.08[ab]
Black Coffee	3.50±0.20[ab]	129.00±2.00[ab]	52.00±2.00[b]	26.00±2.00	6.10±0.17[a]	125.00±1.00[ab]
Positive Control	4.30±0.20[b]	135.00±1.73[b]	54.00±2.00[b]	30.00±2.64	4.30±0.26[b]	90.00±2.00[b]

Results are expressed as Mean ± Standard Deviation, n=3, values with different superscript are statistically different at ($P < 0.05$). Values with (a) superscript are significantly different from Positive control at $p < 0.05$ and values with (b) superscript are significantly different from Negative control at $p < 0.05$.

Table 5: Effect of Green Tea and Black Coffee on Electrolytes, Urea and Creatinine Levels After Third Week Of Treatment.

Sample	Potassium (mmol/L)	Sodium (mmol/L)	Chloride (mmol/L)	Bicarbonate (mmol/L)	Urea (mmol/L)	Creatinine (µmol/l)
Negative Control	3.40±0.20[a]	123.33±1.52[a]	54.00±2.64[a]	32.00±1.00	6.60±0.10[a]	130.00±1.00[a]
Green Tea	3.10±0.10[a]	117.00±3.00[a]	51.00±1.00[ab]	28.00±2.00	6.20±0.20[ab]	127.00±1.00[ab]
Black Coffee	3.40±0.36[ab]	128.00±1.73[a]	53.00±3.46[ab]	30.00±4.35	6.50±0.25[b]	128.00±2.00[b]
Positive Control	5.80±0.40[b]	158.00±19.00[b]	79.00±2.00[b]	26.00±2.00	7.70±0.10[b]	155.00±2.00[b]

Results are expressed as Mean ± Standard Deviation, n=3, values with different superscript are statistically different at ($P < 0.05$). Values with (a) superscript are significantly different from Positive control at $p < 0.05$ and values with (b) superscript are significantly different from Negative control at $p < 0.05$

HISTOPATHOLOGICAL RESULTS

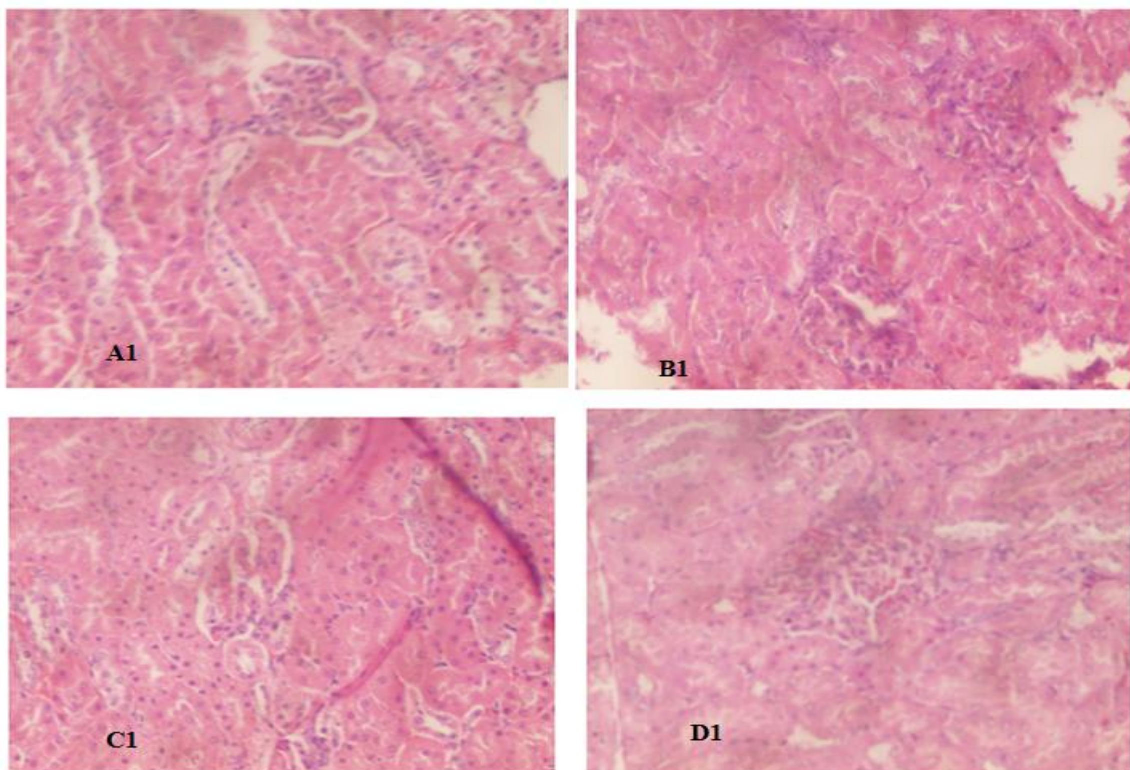

Fig 1: Kidney Histology After One Week Of Treatment.

KEY
A1 = Negative Control
B1 = Green Tea
C1 = Black Coffee
D1 = Positive Control

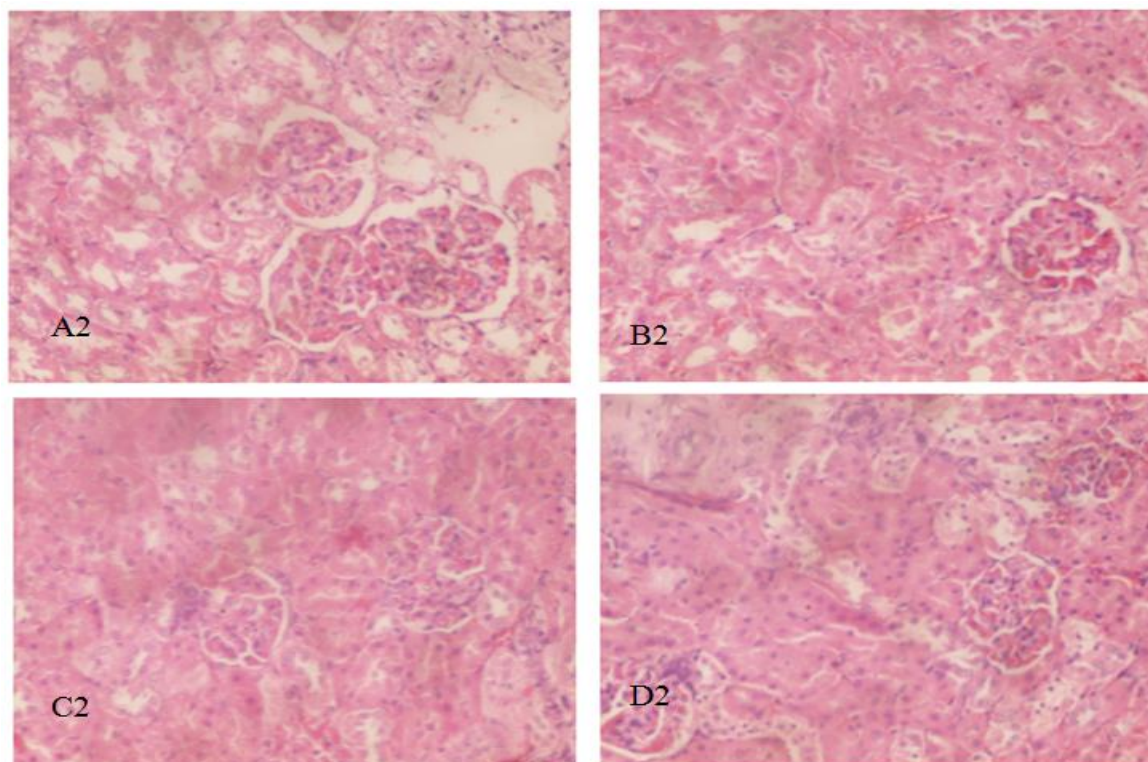

Fig 2: Kidney Histology After Second Week Of Treatment.

KEY
A2 = Negative Control
B2 = Green Tea
C2 = Black Coffee
D2 = Positive Control

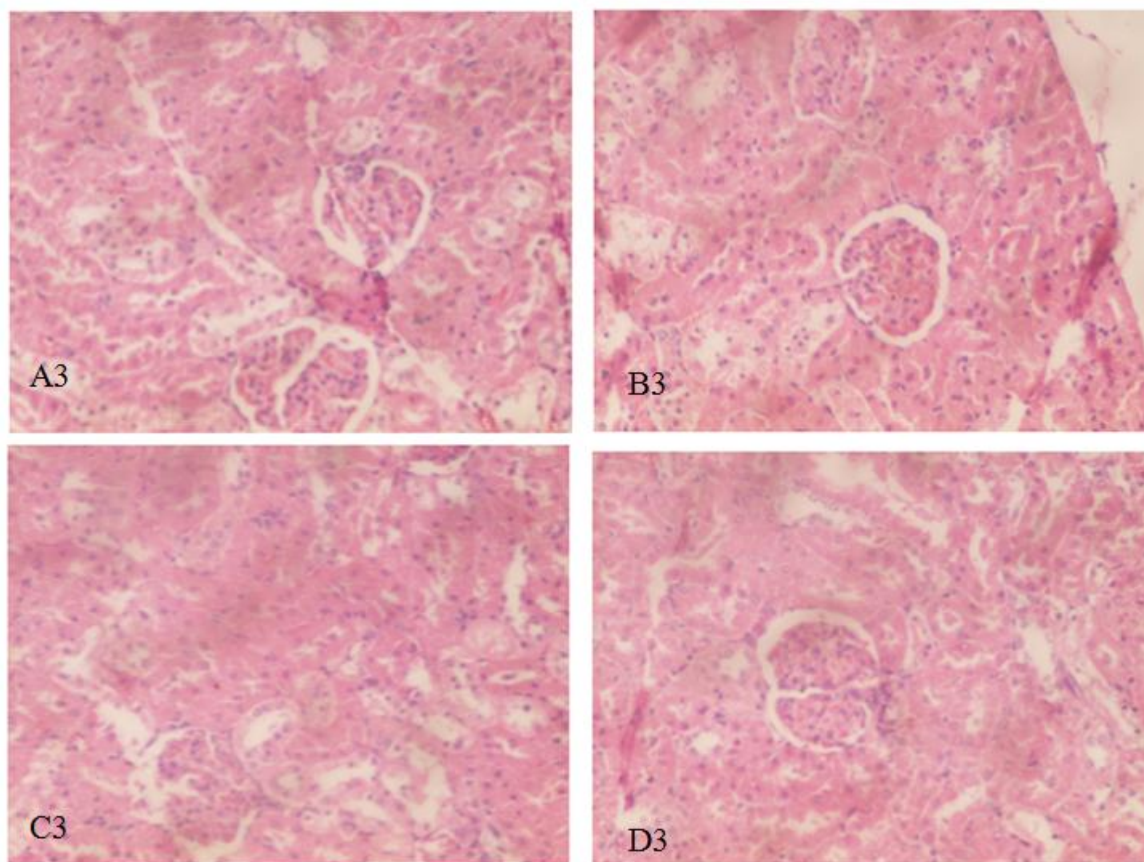

Fig 3: Kidney Histology After Third Week Of Treatment.

KEY
A3 = Negative Control
B3 = Green Tea
C3 = Black Coffee
D3 = Positive Control

DISCUSSION

Natural compounds derived from herbs can be of medicinal use. One of such natural compounds that has been extensively investigated is epigallocatechin-3-gallate (EGCG), a major polyphenol found in the tea plant (*Camellia sinensis*). A growing body of recent evidence has shown that EGCG may be a promising therapeutic or protective agent in various kidney diseases (Rattiyaporn and Visith, 2019). Hyperglycemic state triggers oxidative stress and inflammation mediated by altered metabolic pathways in a self-perpetuating cycle, promoting progression of cell injury and of end-stage Kidney disease (Rayne *et al.,* 2019).

Hyperglycemia is blood glucose greater than 6.9mmol/L while fasting. Hyperglycemia if untreated for long periods of time, could lead to damage of tissues and organs (American Diabetes Association, 2020). The observed glucose level from this research rose from 5.2 ± 0.20 mmol/L to 7.1± 0.16 mmol/L after being induced, on treatment with green tea and black coffee the glucose level dropped to 4.3 ± 0.10 and 4.1± 0.20 respectively after 3 weeks of treatment. This shows that green tea and black coffee are very effective in maintaining and stabilizing blood glucose level.

Drinking excessive amounts of green tea has been reported to cause symptomatic hypokalaemia (low serum potassium) in those taking concomitant medications. It was reported by Fukumoto et al (2013) that a 49-year-old Japanese man developed hypokalaemia myopathy after consuming large quantities of green tea alongside chronic consumption of a cold remedy. This effect was attributed to caffeine, which is present in high concentrations in green tea and has been shown to induce hypokalaemia (Gennari, 1998). Potassium is one mineral

that plays an important role in controlling the amount of fluid in the body. Normal potassium level in the blood ranges from 3.6 mmol/L to 5.2 mmol/L (Sabrina, 2019). From the studies, after being induced the level of Potassium was at 5.10±0.26 mmol/L, after treatment with green tea the level was at 3.30±0.10 mmol/L for the first week, the rat treated with black coffee had a blood potassium level of 3.50±0.43 mmol/L all for the first week. For the second week the level of blood potassium for the rat induced was at 6.00±0.26 mmol/L, the rat treated with green tea had a blood potassium level of 4.50±0.10 mmol/L and the rat treated with black coffee had a blood potassium level of 3.50±0.20 mmol/L. In the third week, the blood blood potassium level rapidly dropped to 3.40±0.20 mmol/L for the induced rat, the rats treated with green tea and black coffee had blood potassium levels of 3.10±0.10 mmol/L and 3.40±0.36 mmol/L respectively.

Due to their high catechin and caffeine content (Mica *et al.,* 2021) green tea and black coffee influences the levels of sodium, chloride and Bicarbonate in blood serum. There is a significant decrease in the blood sodium level when treated with green tea from 155.00±13.74 mmol/L to 123.00±14.73 mmol/L and 131.00±6.08 mmol/L when treated with black coffee in the first week of treatment. In the second week of treatment there's is also a significant decrease in the level of sodium from 165.00±2.64 mmol/L to 135.00±2.64 mmol/L and 129.00±2.00 mmol/L on treatment with green tea and black coffee respectively. On the third week of treatment the level of Sodium decreased from 123.33±1.52 mmol/L to 117.00±3.00 mmol/L on treatment with green tea and 128.00±1.73 mmol/L on treatment with black coffee.

In the first week of treatment, the level of chloride observed in the positive control was 56.00±1.73 mmol/L, the inducement increased the level to 61.00±4.00 mmol/L, this level was reduced to 52.00±1.00 mmol/L on treatment with green tea and on treatment with black coffee it went to 53.00±1.73 mmol/L. In the second week the level of chloride in the induced rat stood at 65.00±1.00 mmol/L, this value decreased on treatment with black coffee to 52.00±2.00 mmol/L but there was more decrease on treatment with Green Tea to 50.00±1.73 mmol/L, the level for the positive control was 54.00±2.00 mmol/L.

The chloride level on the third week of treatment with green tea and black coffee was 51.00±1.00 mmol/L and 53.00±3.46 mmol/L, respectively in each rats which decreased from 54.00±2.64 mmol/L for the induced animal.

The level of Bicarbonate in the induced animal on the first week of treatment was at 22.00±2.00 mmol/L, there was an increase on treatment with green tea to 24.00±2.00 mmol/L and 26.00±2.64 mmol/L on treatment with black coffee.

After the third week of treatment, there was a decrease of 28.00±2.00 mmol/L on treatment with green tea and 30.00±4.35 mmol/L on treatment with Black Coffee when compared to 32.00±1.00 of the induced animals. This shows that green tea and black coffee decreases the level of bicarbonate in blood.

Urea and creatinine Levels stand as markers for kidney health. It has been documented that green tea and its catechin components improve renal failure and inhibit the growth of mesangial cells (Waleed et al, 2008) and this in turn affects the level of urea and creatinine.

Urea levels were seen to be slightly reduced after the first of week of treatment with green tea and black coffee, and the reduction continued after the third week of treatment.

Creatinine levels were also observed throughout the research, creatinine levels after the first week of treatment reduced significantly, on treatment with green tea and black coffee.

After the second week of treatment with green tea and black coffee, the level of creatinine levels also reduced significantly and the level of creatinine continued to decrease on treatment with green tea and black coffee after the third week of treatment.

Upon treatment with green tea on the third week of treatment, urea level had a decrease of 6.20±0.20 mmol/L when compared to negative control 6.60±0.30 mmol/L. On treatment with black coffee on the third week of treatment, urea level had a decrease of 6.50±0.25 mmol/L when compared to the negative control 6.60±0.30 mmol/L. Furthermore, upon treatment with green tea on the third week of treatment, creatinine level had a decrease of 127.00±1.00 µmol/l when compared to negative control 130.00±1.00 µmol/l. On treatment with black coffee on the third week of treatment, creatinine level had a decrease of 128.00±1.00 µmol/l when compared to the negative control 130.00±1.00 µmol/l.

High glucose levels coupled with high blood pressure can accelerate the damage to the blood vessels in the kidneys. This can lead to a drop in kidney function. In medicine, histopathology is the branch of histology that includes the microscopic identification and study of diseased tissue (Robert and Brelje 2014; Leeson *et al.,* 1981). The histology of the kidney was observed after being induced and after treatment, and was compared to a normal kidney.

In the first week of treatment, the rat induced with hyperglycemia showed normal kidney histology. Normal kidney histology were also observed in groups treated with green tea and black coffee, but is distorted in the group not induced or treated showing enlarged glomerular taft and occluded Bowman's capsule.

In the second week of treatment, the animal induced with hyperglycemia and the animal treated with Green Tea showed normal histology, but the animals treated with black coffee showed a mildly distorted kidney, a partly enlarged glomerular taft and marked decrease in Bowman's capsular spaces. The rats not induced or treated showed a histologically distorted kidney with inflammatory cells in the interstitial tissue, there was also a collapse of renal tubules and vacuolation of cytoplasm of the renal tubular epithelial cells.

CONCLUSION

This research showed that green tea and black coffee significantly reduced sugar levels in hyperglycemic Wistar rats. The research also showed that green tea and black coffee decreased the levels of electrolytes, urea and creatinine therefore, this research showed that drinking of green tea and black coffee in moderation and not for prolonged period of time improves general wellbeing.

Competing Interests

Authors have declared that no competing interests exist.

ETHICAL APPROVAL

This research work was carried out with the approval of the University of Port Harcourt research ethics committee.

REFERENCES

1. Agha, I. (2003). BUN. MedlinePlus Medical Encyclopedia (3), 1-2.
2. American Diabetes Association. (2020). Hyperglycemia (12), 2
3. Bakalar, N. (2006). "Coffee as a Health Drink? Studies Find Some Benefits". The New York Times.
4. Bunker, M. L., McWilliams, M. (1979). "Caffeine content of common beverages". Journal of the American Dietetic Association, 74(1): 28-32.
5. Blann, A. (2014) Routine blood tests 1: why do we test for urea and electrolytes? Nursing Times, 110(5): 19-21.
6. Cabrera, C., Artacho, R., & Gime´nez, R. (2006) Beneficial effects of green tea—a review. J Am Coll Nutr, 25: 79–99
7. Cappelletti, S., Piacentino, D., Daria, P., Sani, G., Aromatario, M., (2015). "Caffeine: cognitive and physical performance enhancer or psychoactive drug?". Current Neuropharmacology, 13(1): 71-88.
8. Ciara, C. (2021). 10 Green Tea Benefits and the Best Way to Drink It. Lifehack.
9. Cynthia, S. (2020).10 Health Benefits of Green Tea, According to a Nutritionist. Health.
10. Fukumoto, M., Yamashiro, N., & Kobayashi, F. (2013). A case of hypokalemic myopathy induced by excessive drinking of a beverage containing green tea extract. Clinical Neurol, 53: 239.
11. Gennari, F. J. (1998) Hypokalemia. N Engl J Med, 339: 4518.
12. Harold, N. G. (1992). Green tea composition, consumption, and polyphenol chemistry. Preventive medicine, 21(3): 334-350.
13. Hazel, B. M., George, W. L., & Kenneth, D. F. (1999). Medicinal herbs in the United States: research needs. Environmental health perspectives, 107(10): 773-778.
14. Henry R.F., (1974). Clinical Chemistry Principles and Technics, 2nd Ed., Harper and Row, Hagerstein, M.D., Joseph, C. (2000). Medicinal herbs: drugs or dietary supplements. Biochemical pharmacology, 59(3): 211-219.
15. Khan, N., & Mukhtar H. (2013). "Tea and health: studies in humans". Current Pharmaceutical Design (Literature Review), 19(34): 6141–7.
16. Koo, M., & Cho, C. (2004). Pharmacological effects of green tea on the gastrointestinal system. Biology, Medicine. European journal of pharmacology, 7: 23.
17. Kris, G. (2020). 10 Evidence-Based Benefits of Green Tea. Healthline.
18. Lacey, B. (2021). 10 Benefits of Green Tea | Why You Should Drink it Every Day. Health & Wellness.
19. Leeson, T. S., Leeson, C. and Roland (1981). Histology. W. B. Saunders Company, 4: 600.
20. Levinson, S.S (1976). Clin Chem, 22: 273.
21. Maruna, R. F. L. (1958). Clin. Chem Acta, 2: 581.
22. McKay, D. L., and Blumberg, J. B. (2002) The role of tea in human health: An update. J Am Coll Nutr. 21:1-13.
23. Megan, W. (2021). What are the health benefits of green tea?. Medical News Today.
24. Mica, C., Faizah, T., Alendre, L., Quyen, D., Tyeshia, S., & Monika, S. (2021). Effect of Water Hardness on Catechin and Caffeine Content in Green Tea Infusions. Molecules, 26(12): 3485.
25. Naghma, K., and Hasan, M. (2007). Tea polyphenols for health promotion. Life Sciences, 81: 519-533.
26. Pauss, A., Roza, A., Ledrut, M. J., Naveau, H., and Nyns E. J. (1990). Bicarbonate determination in complex acid-base solutions by a back-titration method, Environmental Technology, 11(5): 469-476.
27. Poole, R., Kennedy, O. J., Roderick, P., Fallowfield, J. A., Hayes, P. C., & Parkes, J. (2017). "Coffee consumption and health: umbrella review of meta-analyses of multiple health outcomes". BMJ, 359: 5024.
28. Rattiyaporn, K., & Visith, T. (2019). Protective effects of epigallocatechin-3-gallate from green tea in various kidney diseases. Advances in Nutrition, 10(1): 112-121.
29. Rayne, G. A., Glaucevane da Silva, G., Sandra, M. L. V., & Juliana, C. S. (2019). Kidney disease in diabetes mellitus: cross-linking between hyperglycemia, redox imbalance and inflammation. Arquivos brasileiros de cardiologia, 112: 577-587.
30. Robert, L. S., and Brelje, C. T. (2014). A Guide to Microscopic Structure of Cells, Tissues and Organs. Atlas of Human Histology.

31. Sabu, M. C., Priya, T. T., Ramadasan, K., & Ikuo, N. (2010). Beneficial effects of green tea: a literature review. Chinese medicine, 5(1): 1-9.
32. Sabrina, F. (2019). What Is a Potassium Blood Test? WebMD, 2(2): 21.
33. Tietz NW. (1995). Clinical Guide to Laboratory Tests, 3rd Edition (US, Philadelphia:W.B. Saunders).
34. Tim, J. (2018). Kidney Overview. Heathline, 14(1): 2.
35. Trinder, P. (1951). Analyst, 76: 596.
36. Shahin, S. A., Naresh, K., Abhinav, L., Angad, S., Hallihosur, S., Abhishek, S., Utpal, B. (2008). Indian medicinal herbs as sources of antioxidants. Food research international, 41(1): 1-15.
37. Sumit, B., Navneet, S., Pooja, M., & Shivani, C. (2011). Pharmacological profile of green tea and its polyphenols: A review Article, 14(2): 110-111.
38. Waleed, M. R., Suad, A., Mousa A., & Sami, A. (2008). Effect of green tea on kidney tubules of diabetic rats. Effect of green tea on kidney tubules of diabetic rats. British journal of nutrition, 100(3): 652-659.
39. Walter, F. B. (2005). Medical Physiology: A Cellular And Molecular Approach. Elsevier/Saunders, 22(7): 837.
40. Williams, R. J., Spencer, J. P. E., & Rice-Evans, C. (2004). "Flavonoids: Antioxidants or signaling molecules?". Free Radical Biology and Medicine, 36(7): 38-49.