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Histopathological Of Goko Cleanser (Herbal Mixture) On The Kidney Of Adult Female Wistar Rats

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Abstract: ***Over the years, medicinal plants have shown to posses some biological constituents that significantly affect man when taken into the body system. This study was carried out to evaluate the histopathological effects of Goko Cleanser herbal mixture on the kidney of adult female Wistar rats. A total of twenty five animals weighing 160g - 280g were used. The rats were randomly selected into five groups of five (5) each. The groups were designed as group 1-5. Group 1 served as control group, group 2 took 1000mg/kg of the herbal mixture, group 3 took 1500mg/kg of the herbal mixture, group 4 took 2000mg/kg and group 5 took 4000mg/kg. The phytochemical test reveals that Goko Cleanser herbal mixture possesses tannin, saponin and flavonoids. The food intake was measured daily. Result from food intake at the first week 1 revealed that there was no significant (P>0.05) increase in the test groups when compared to the control group. In week 2, there was a significant (P<0.05) decrease in group 4 and 5, while there was no significant (P>0.05) increase in group 2; and no significant (P>0.05) decrease in group 3 compared to the control groups respectively. In week 3, there was a significant (P<0.05) decrease in food intake in the treated groups when compared to the control group; and there was a significant (P<0.05) decrease in body weight in groups 2, 4 and 5 while group 3 had no significant (P>0.05) decrease. The histopathological results reveal mild to moderate haemorrhage on the interstitium of the kidney. The possible mechanism of action could be as a result of some of its phytochemical constituents which cause a break in the membrane of cellular structure of the kidney leading to lipid peroxidation. This further increases the kidney enzymes which then serve as biomarkers for organ damage. Hence, the results from this study show that Goko Cleanser herbal mixture possesses dose-dependent hepatotoxic (haemorrhagic) effects.***

I. INTRODUCTION

In Nigeria, herbal mixtures have been the strength of traditional medicine for years now. In recent times, many authors have conducted several research works on herbal mixtures to determine its therapeutic effects (Udochukwu *et al.*, 2015; Amandeep *et al*., 2015; Huzaifa *et al*., 2014; Feng *et al*., 2014; Gazuwa *et al*., 2013; Ekor, 2013; Akinjogunla *et al.*, 2011; and Nnodim *et al*., 2010). Over three quarter of the world's population is using herbal medicine with an increasing trend globally. In addition, herbal medicine may be beneficial but not completely harmless (Oreagba *et al*, 2011).

Goko cleanser is a herbal mixture used for the treatment of various kinds of diseases and infections. Its contents include: *Vernonia amygdalina, Cajanus cajan, Zingiber officinale, Allium sativum, Saccharum officinarum, Caramel*.

Scientist has conducted individual researches on the above herbal content or a combination of two to determine their positive or negative effect. However arguments have been raised on the combination of these various herbs into a herbal mixture. *Vernonia amygdalina* one of the herbal content of the mixture is a member of the *Asteraceae* family, is a small shrub that grows in tropical Africa. It is commonly called Bitter leaf in English because of its bitter taste and it is locally known as Onugbu in Igbo. The infusion of the leaf

induces the haemolysis of mammalian erythrocyte in vitro with Human-SS having the highest susceptibility (Akinjogunla *et al.*, 2011; and Udochukwu *et al.*, 2015).

Cajanus cajan commonly called pigeon pea, is a perennial legume from the family fabaceae. Its seeds have become a common food grain in Asia, Africa and Latin America. It is a major source of protein. In combination with cereals, pigeon pea makes a well balanced human food. It is locally called Otili in Yoruba .The glycemic profile of the aqueous extract of Cajanus cajan leaves significantly increases the fasting blood glucose levels of normal rats (Pal *et al.*, 2011).

Zingiber officinale is commonly called ginger and locally known as Atale in Yoruba. It is a flowering plant, in the family Zingiberaceae whose rhizome, ginger root or simply ginger, it is widely used as a spice or a folk medicine. It is a herbaceous perennial which grows annual 9m tall bearing narrow green leaves and yellow flowers. If consumed raw in large amounts, it causes intestinal blockage and inflammatory bowel disease in people with gastric ulcers (Nnodim *et al*., 2010).

Allium sativum is commonly known as garlic and locally called Ata in Yoruba and Tafarnuwa in Hausa. It is a specie in the onion genus – allium. Its close relative includes onion and shallot. It was known to ancient Egyptians and has been used for both culinary and medicinal purposes. It adversely causes haemorrhaging if consumed with prescribed anticoagulants. It may also cause menstrual irregularities (Huzaifa *et al*., 2014; and Gazuwa *et al*., 2013).

Saccharum Officinarum is commonly known as sugar cane locally called Okpete in Igbo, Ireke in Yoruba and Rake in Hausa. It causes excessive urination and indigestion when consumed in large quantity (Amandeep *et al*., 2015; and Feng *et al*., 2014).

The kidney is a urinary organ, ovoid in shape and reddish brown in colour. It measures approximately 10cm in length, 5cm in width and 2.5cm in thickness. The kidney functions to remove excess water, salt and waste of protein metabolism from the blood while returning nutrients and chemicals to the blood (Moore and Dalley, 2006). The kidney is the main site for diabetes and renal cysts which can lead to kidney failure. Unhealthy herbal mixtures can cause kidney disease.

Different herbs have been found to be useful to humans as they are used in the treatment of various ailments, however most of these have been abused by individuals or organizations that produce different herbal mixtures and sell them with different claims of efficacy. Most of these claims may be unverified and thus could pose serious threats to the general human health and body functions. Though these herbal mixture is widely used in the treatment of various ailments around the world, there is still no knowledge on its exact effect on various body structures and functions. Therefore, this study aims at investigating the specific histopathological effect of this herbal mixture (Goko Cleanser) on the kidney using female Wistar rats as experimental models.

II. MATERIALS AND METHODS

LOCATION OF THE STUDY

This study was carried out in the animal house of the Department of Anatomy, Nnamdi Azikiwe University, Nnewi Campus, Anambra State, Nigeria. The animals procured from Sanitas Animal Farm, Ekwulumili, were made to acclimatize for a period of two weeks at the animal house of Anatomy department, Nnamdi Azikiwe University, Nnewi campus after which the test substance was administered for a period of 21 days.

MATERIALS

25 Female Wistar rats, Goko Cleanser Herbal Mixture, Standard rat feed, Plastic cages with iron netting, Animal weighing balance, Oral cannula, Sets of EDTA treated sample bottles, 10ml syringe (Disposable), Distilled water, Latex gloves and Cotton wool

EXPERIMENTAL ANIMALS

The experimental animals were twenty five adult female Wistar rats weighing between 160-280g. The rats were differentiated by colour marks peculiar to each group. They were kept in plastic cages with iron netting in standard conditions and fed properly with normal growers' mesh which was produced by Premier Feed Mills Co. Limited (A subsidiary of Flour Mills Nigeria Plc). The rats were divided into five groups, with group 1, 2, 3, 4 used as the test group while Group 5 served as the control group. All rats were weighed prior to the commencement of administration and subsequently weighed weekly (once a week) using animal weighing balance (CAMRY IILBXOZ).

COLLECTION AND IDENTIFICATION OF THE HERBAL MIXTURE

The herbal mixture (Goko cleanser) was purchased from a pharmacy shop around Nnamdi Azikiwe University Teaching Hospital Nnewi. Phytochemical Analysis was carried out to determine the components of the herbal mixture at the Pharmacological Laboratory in Nnamdi Azikiwe University, Agulu Campus.

PHYTOCHEMICAL ANALYSIS OF THE HERBAL MIXTURE (GOKO CLEANSER)

This includes both the quantitative and qualitative analysis of the herbal mixture Goko cleanser.

QUALITATIVE ANALYSIS

- ✓ Saponin – Moderately present
- ✓ Tannin – Trace / Mildly present
- ✓ Flavonoid – Trace / Mildly present
- ✓ Alkaloid, steroid, Tapernoid, cardiac glycoside, protein, and carbohydrate are all negative.

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Chemical tests were carried out on the aqueous extract and powdered specimens using standard procedures to identify constituents as described by Solowara (1993), Trease (1989) and Harborne (1973).

TEST FOR SAPONINS

About 2g of the powdered sample was boiled in 20ml of distilled water in a water bath and then filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion

TEST FOR TANNINS

About 0.5g of the dried powdered sample boiled in 20ml of water in a test tube and then filtered a few of 0.1% ferric chloride was added and observed for brownish or a blue black colouration.

TEST FOR FLAVONOIDS

Three methods were used to determine the presence of flavonoids in the plant sample (Solowara, 1993; Harborne, 1973).

- ✓ 5ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing.
- ✓ Few drops of 1% aluminium solution were added to a portion of each filtrate .A yellow colouration was observed indicating the presence of flavonoids.
- ✓ A portion of the powdered plant sample was in each case heated with 10ml of ethyl acetate over a steam bath for 3min.The mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration was indicating a positive test for flavonoids.

TEST FOR STEROIDS

2ml of acetic anhydride was added to 0.5g ethanolic extract of each sample with 2ml H_2SO_4. The colour changed from violet to blue or green in some samples indicating the presences of steroids.

TEST FOR TERPENOIDS (SALKOWSKI TEST)

5ml of each extract was mixed in 2ml of chloroform, and 3ml concentrated H_2SO_4 was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

TEST FOR CARDIAC GLYCOSIDES (KELLER-KILLANI TEST)

5ml of each extracts was treated in 2ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated sulphuric acid. A brown ring of the interface indicates deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

III. QUANTITATIVE ANALYSIS

ALKALOID DETERMINATION USING HARBORNE (1973) METHOD

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

TANNIN DETERMINATION BY VAN-BUVEN AND ROBINSON (1981) METHOD

500mg of the sample was weighed into a 50ml plastic bottle. 50ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50ml volumetric flask and made up to the mark. Then 5ml of the filtered solution was pipetted out into a test tube and mixed with 2ml of 0.1M $FeCL_3$ in 0.1NHCl and 0.008M potassium Ferro-cyanide. The absorbance was measured at 120nm within 10 minutes.

SAPONIN DETERMINATION

The method used was that of Obadoni and Ochuko (2001). The samples were ground and 20g of each were put into a conical flask and 100cm of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4hours with continuous stirring at about 550c.The mixture was filtered and the residue re-extracted with another 200ml 20% ethanol. The combined extracts were reduced to 40ml over water bath at about 90^0C. The concentrate was transferred into a 250ml separatory funnel and 20ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60ml of n-butanol was added. The combined n-butanol extracts were washed twice in 10ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the samples were dried in the oven to a constant weight, the saponin content was calculated as percentage

FLAVONOID DETERMINATION BY THE METHOD OF BOHM AND KOLPAL-ABYAZAN (1994)

10g of the plant sample was extracted repeatedly with 100ml of 80% aqueous methanol at room temperature. The

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whole solution was filtered through Whatman filter paper number 42 (125mm).The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

RESULT OF PHYTOCHEMICAL ANALYSIS

Phyto-constituents	Goko Cleanser herbal mixture
Saponin	++
Flavonoids	+
Tannin	+
Alkaloid	-
Steroid	-
Cardiac glycoside	-
Protein	-
Carbohydrate	-

++ (Moderately present), + (Mildly present), - (Absent)

TOXICITY TEST OF GOKO CLEANSER (CALCULATION OF LD50) ACUTE TOXICTY STUDY

The median lethal dose (LD_{50}) of Goko Cleanser herbal mixture was carried out in the Department of Anatomy, Faculty of Basic Medical Science, Nnamdi Azikiwe University, Nnewi. This was determined using the modified method of Dietrich Lorke (1983). In this study, a total of 13 rats were used. They received the extract via oral route and it was carried out in two phases.

PHASE I

Nine (9) rats were used and they were grouped into three made of three adult Wistar rats.

Group 1 received 10mg/kg
Group 2 received 100mg/kg
Group 3 received 1000mg/kg

The animals were observed over a period of 24hrs for mortality. From the result of phase 1, the second phase was carried out.

PHASE II

Group 1 received 1200mg/kg
Group 2 received 2600mg/kg
Group 3 received 3900mg/kg
Group 4 received 5000mg/kg

The animals were monitored over a period of another 24hrs for mortality. The median lethal dose was obtained at this phase.

PHASE	DOSE	DEATH	OBSERVATION
1	10mg/kg	0/3	Nil
	100mg/kg	0/3	Nil
	1000mg/kg	0/3	Nil
2	1200mg/kg	0/1	Nil
	2600mg/kg	0/1	Calm and no death occurred
	3900mg/kg	1/1	Died within 48 hours
	5000mg/kg	1/1	Died within 24 hours

$LD_{50} = \sqrt{a \times b}$
A = Maximum dose with 0% mortality (2600mg/kg)
B = Minimum dosed with 100% mortality (3900mg/kg)
LD_{50} *of Goko Cleanser* $= \sqrt{2600 \times 3900} = 3184.34$*mg/kg*
LD_{50} *of Goko Cleanser = 3184.34mg/kg*

PREPARATION OF STOCK SOLUTION

200ml of Goko Cleanser was oven dried at 50ºC and the concentrated Goko cleanser was measured to be 5g.

5g of Goko cleanser was dissolved in 100mls of distilled water to get a stock solution 50mg/ml.

1g = 1000mg
5g = 5000mg = 5000mg/100ml
Stock Solution =50mg/ml

$$\text{Using} = \frac{\text{Weight of Animal} \times \text{Dose [kg]}}{\text{Stock}}$$

DRUG ADMINISTRATION

The drugs were administered to the rats in the test group orally using an oral cannula with rubber tubing, while rats in the control group received distilled water and grower feed. The extracts were administered once daily within the hours of 08:00am and 09:00am. All rats in both control and test group were allowed free access to food and water, throughout the experimental period.

EXPERIMENTAL PROTOCOL

Before administration, the rats were weighed and their weight ranged between 160-320g.They were then divided into five experimental groups according to their body weight from highest to lowest with five in each group labelled Group 1-5. The experimental groups 2-5 received different doses of drug as follows:

- ✓ Group 1 – Control
- ✓ Group 2 – Received 1000mg/kg of Goko cleanser
- ✓ Group 3 – Received 1500mg/kg of Goko cleanser
- ✓ Group 4 – Received 2000mg/kg of Goko cleanser
- ✓ Group 5 – Received 4000mg/kg of Goko cleanser.

COLLECTION OF ANIMAL ORGANS

The experimental animals were anaesthetized by chloroform inhalation, followed by cervical dislocation and the animals were dissected and the kidneys were harvested and put in normal saline to maintain physiological conditions after which they fixed in 10% formalin.

PRECAUTIONS

- ✓ This study ensured sterility of instruments throughout the experiment.
- ✓ This study ensured that each organ after harvesting was properly rinsed in normal saline, and then properly fixed in 10% formalin.

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TISSUE PROCESSING

After weighing the organ, the kidney was immediately fixed in 10% formal saline in order to preserve the various constituents of the cell in their normal micro anatomical positions and to prevent autolysis and putrefaction.

Tissue sections were produced through normal histological methods of dehydration, clearing, impregnation, embedding, sectioning and staining (with H &E) after fixation. The micrographs of the relevant stained sections were subsequently taken with the aid of a light microscope.

FIXATION

The essence of fixation is to preserve the various constituents of the cells in their normal micro anatomical position and to prevent autolytic changes and putrefaction. The tissues for this experiment were fixed in 10% formalin solution for about 10hours.

DEHYDRATION

The tissues after fixation were dehydrated by putting them in ascending grades of alcohol for 1-2 hours; this is to remove water from the cells of the tissue which are not miscible with the embedding agent (paraffin wax).

CLEARING

Clearing is the process of removing alcohol from the tissue and replacing it with a clearing agent which is miscible with both alcohol and paraffin wax (clearing agent), for this experiment Xylene was used as the clearing substance. The tissues were placed were placed in xylene for a period ranging from 1-2 hours (this is to avoid over exposure of the tissue to the clearing agent which may cause it to become brittle).

IMPREGNATION

This stage is also called infiltration and involves the process of replacing a clearing agent with molten paraffin wax. At this stage, the tissues were placed in molten paraffin wax at a temperature of about 35ºC (3ºC above the melting point of paraffin wax) and were passed through two changes of paraffin wax in the oven, 4hours for each time. After that, the tissues were removed from the paraffin wax.

EMBEDDING

Embedding is a process of burying a tissue in molten paraffin wax, the paraffin wax becomes a firm structure that forms a support medium for the tissue during microtomy. Metal mould was sprayed with mould release fluid. The mould was then filled with molten paraffin wax and the tissue placed in it immediately with forceps, with the face to be cut facing downwards. The tissue was embedded with its identifying number written on the projecting tag. When the paraffin wax cooled, thin scum of solid paraffin was formed on the bottom of the metal blocks, after which it was immersed in water it solidified and was then removed ready for sectioning.

SECTIONING

Sectioning is also called microtomy; it is a process of cutting thin sections from tissue with a precision machine called microtome. The block was fixed in the block holder of the microtome and was ensured that it was secure, and then the excess wax was trimmed from the block surface to expose the tissue. Once the tissue was revealed an adjustable knife angle was set and then the thickness of the gauge was rest to 5 micrometer and the cutting began.

ATTACHING PARAFIN WAX SECTIONS TO SLIDES

A slice of the section was taken and one side was sticky by rubbing starch. The section was put in the centre of the slide and 40% alcohol was flooded on the slide in such a way that the section started floating, the section was immersed in water bath keeping the temperature between 50-55ºC so that the section became straightened and wrinkles disappeared. Water was drained off and the slide put in an incubator at 37ºC overnight so that the section was completely fixed on the side and became dry.

STAINING

Dyes are used to give contrasting colours to different elements of the cells or tissues to make them conspicuous. Haematoxylin and Eosin dye was used in this experiment.

The slide was deparaffinize in xylene for 1 minute. It was then immersed in absolute alcohol for 30 seconds. Then it was immersed in descending grades of alcohol. It was rinsed in water. The slide was then put in aqueous solution of haematoxylin for 5 minutes. The slide was then washed in running tap water for 5 minutes till colour of section becomes blue (this is called bluing). The slide was then immersed in 1% aqueous eosin for 30 seconds. Afterwards the slide was rinsed in water for few seconds. The slide was then immersed in descending grades of alcohol, 30 seconds for each step. The slide was then immersed twice in absolute alcohol for 3 seconds. It was then cleared in xylene for 3 minutes. The slide was then mounted in DPX under a cover slip avoiding air bubbles from getting in.

MOUNTING TECHNIQUE

Sufficient number of cover slip for the section to be mounted was wiped clean of dust with 70% alcohol. Then two drops of mountants was placed on the section which was laid along the middle of the section to minimize the likelihood of trapping air bubbles. The slide was quickly inverted over the cover slip and was then brought down horizontally until the mountant made contact. The cover slip and the slide were attached and finally viewed under the light microscope.

STATISTICAL ANALYSIS

Data were analyzed using Statistical Package for Social Sciences (SPSS) version 16, while experimental results were expressed as mean ± standard error mean (S.E.M) using one way analysis of variance (ANOVA), followed by post hoc

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LSD multiple comparison and values were considered significant at P<0.05.

IV. RESULTS

Food intake		MEAN	±SEM	P-VALUE	F-VALUE
Week 1	GROUP 1(CONTROL)	28.50	±2.98		
	GROUP 2 (1000mg of Goko Cleanser)	29.72	±2.03	0.671	
	GROUP 3 (1500mg of Goko Cleanser)	30.22	±1.64	0.551	0.232
	GROUP 4 (2000mg of Goko Cleanser)	31.12	±1.21	0.368	
	GROUP 5 (4000mg of Goko Cleanser)	29.52	±1.66	0.722	
Week 2	GROUP 1(CONTROL)	30.75	±1.49		
	GROUP 2 (1000mg of Goko Cleanser)	32.07	±2.02	0.529	
	GROUP 3 (1500mg of Goko Cleanser)	29.12	±1.21	0.442	28.130
	GROUP 4 (2000mg of Goko Cleanser)	20.25	±0.32	0.000	
	GROUP 5 (4000mg of Goko Cleanser)	14.25	±1.63	0.000	
Week 3	GROUP 1(CONTROL)	46.50	±3.88		
	GROUP 2 (1000mg of Goko Cleanser)	24.50	±0.64	0.000	
	GROUP 3 (1500mg of Goko Cleanser)	23.87	±0.59	0.000	38.401
	GROUP 4 (2000mg of Goko Cleanser)	20.25	±0.85	0.000	
	GROUP 5 (4000mg of Goko Cleanser)	13.75	±1.65	0.000	

Table 1: Effect of Goko cleanser herbal mixture on food intake after week 1, 2 and 3 of treatment

All data were analyzed using one-way ANOVA and values were considered significant at P<0.05.

The result above revealed that, there was an increase in food intake of the animals in week 1 for all test group 2 (29.72±2.03), 3 (30.22±1.64), 4 (31.12±1.21) and 5 (29.52±1.66) when compared to the control group (28.50±2.98). This increase in food intake was not significant. In week 2, there was a decrease in food intake when comparing the control group (46.50±3.88) with the test group 2 (32.07±2.02), 3 (29.12±1.21), 4 (20.25±0.32) and 5 (14.25±1.63), but the decrease in food intake was significant in group 4 and 5. In week 3, there was a significant decrease in food intake in group 2 (24.50±0.64), 3 (23.87±0.59), 4 (20.25±0.85) and 5 (13.75±1.65) when compared to the control group (46.50±3.88).

		MEAN	±SEM	P-VALUE	T-VALUE
Group 1 (CONTROL)	Initial	200.00	±16.32		
	Final	235.00	±9.57	0.035	-3.656
Group 2 (1000mg of Goko Cleanser)	Initial	210.00	±25.16		
	Final	182.50	±17.01	0.049	3.220
Group 3 (1500mg of Goko Cleanser)	Initial	192.50	±12.50		
	Final	162.50	±4.78	0.069	2.777
Group 4 (2000mg of Goko Cleanser)	Initial	220.00	±11.54		
	Final	162.50	±6.29	0.007	6.734
Group 5 (4000mg of Goko Cleanser)	Initial	255.00	±22.17		
	Final	147.50	±4.78	0.025	4.196

Table 2: Effect of Goko cleanser herbal mixture on the Initial and Final body weight after 21 days of treatment

All values were analyzed using dependent T-test and values were considered significant at P<0.05.

Result from the table above shows that, there was an increase in the body weight in control group when comparing the Initial (200.00±16.32) and Final (235.00±9.57) body weight. This increase was significant. In group 2, there was a decrease in the body weight when comparing the Initial (210.00±25.16) and Final (182.50±17.01) body weight. This decrease was significant. In Group 3, there was a decrease in the body weight when comparing the Initial (192.50±12.50) and Final (162.50±4.78) body weight. This decrease was not significant. In group 4, there was a decrease in the body weight when comparing the Initial (220.00±11.54) and Final (162.50±6.29) body weight. This decrease was significant. In group 5, there was a decrease in the body weight when comparing the Initial (255.00±22.17) and Final (147.50±4.78) body weight. This decrease was significant.

		Mean	±SEM	P-VALUE
Relative kidney weight (g)	Group 1 (Control)	0.60	±0.01	
	Group 2 (1000mg G.C)	0.70	±0.01	0.000*
	Group 3 (1500mg G.C)	0.75	±0.00	0.000*
	Group 4 (2000mg G.C)	0.67	±0.00	0.000*
	Group 5 (4000mg G.C)	0.71	±0.01	0.000*

Table 3: Effect of Goko Cleanser Herbal Mixture on the relative Kidney weight

Result from the table above shows that, there was a significant increase in the relative weight of kidney in group 2 (0.70±0.01), 3 (0.75±0.00), 4 (0.67±0.00) and 5 (0.71±0.01) when compared to that of the control group (0.60±0.01).

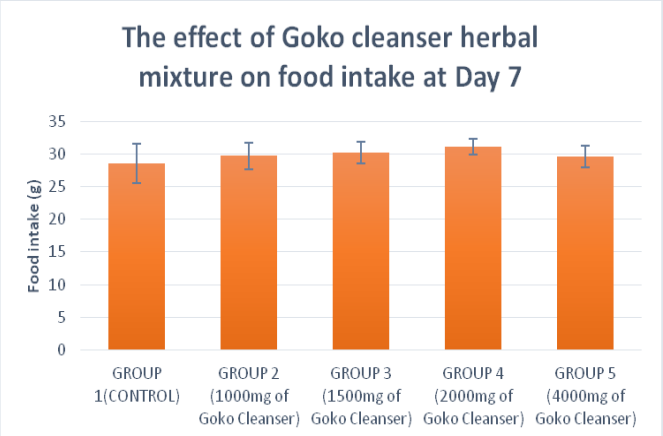

Figure 1: Bar chart showing the effect of Goko cleanser herbal mixture on food intake for week 1

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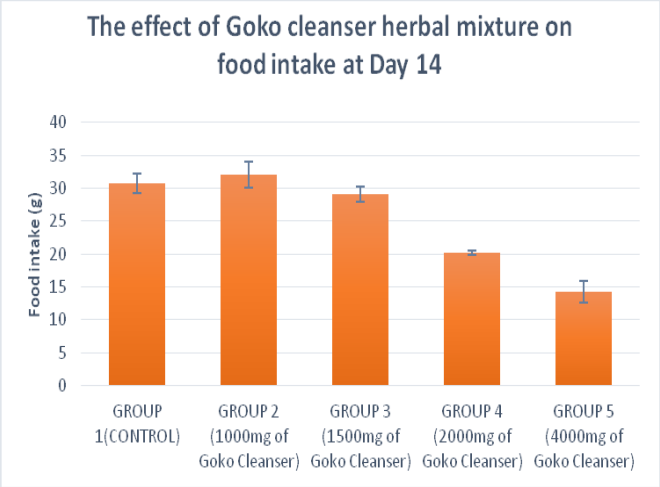

Figure 2: Bar chart showing the effect of Goko cleanser herbal mixture on food intake for week 2

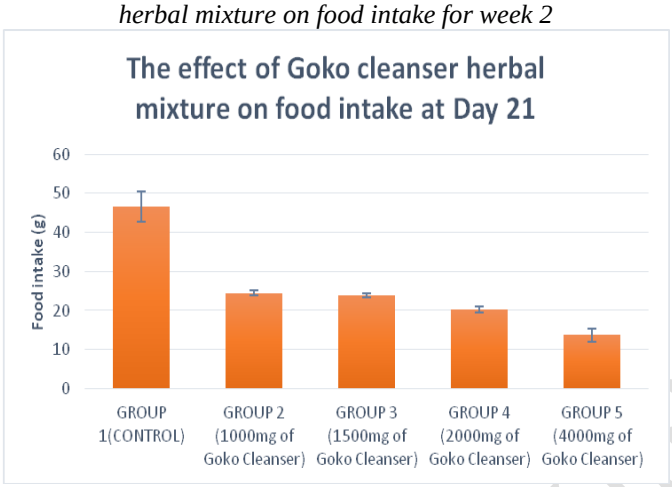

Figure 3: Bar chart showing the effect of Goko cleanser herbal mixture on food intake for week 3

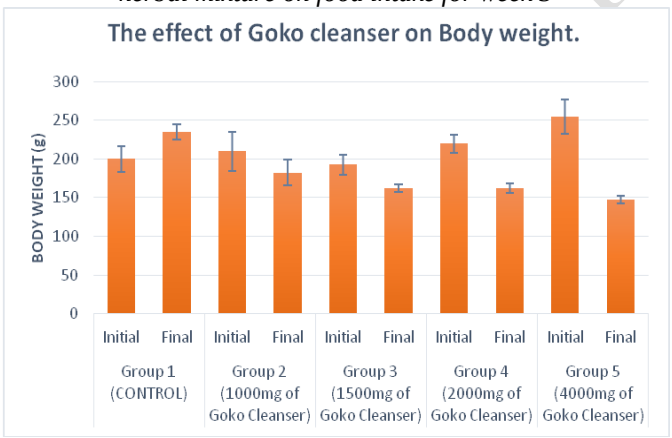

Figure 4: Bar chart showing the effect of Goko cleanser herbal mixture on Body weight

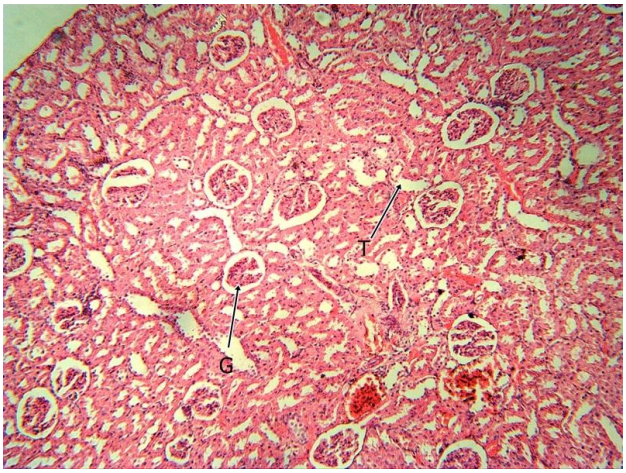

Plate 1: Control

Photomicrograph of renal tissue above shows glomeruli that are evenly distributed, of similar size with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. The interstitium is normal. Stained by H & E technique (X100).

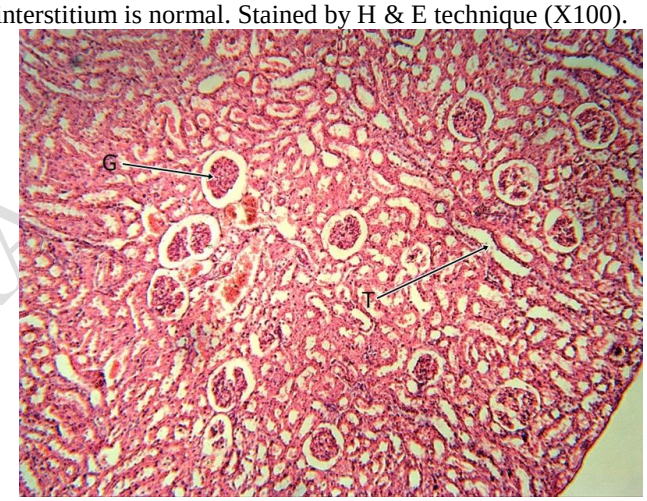

Plate 2: 1000mg Goko cleanser herbal mixture

Photomicrograph above shows glomerular that are evenly distributed, of similar size, with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. There is mild to moderate haemorrhage into the interstitium.

Stained by H & E technique (X100).

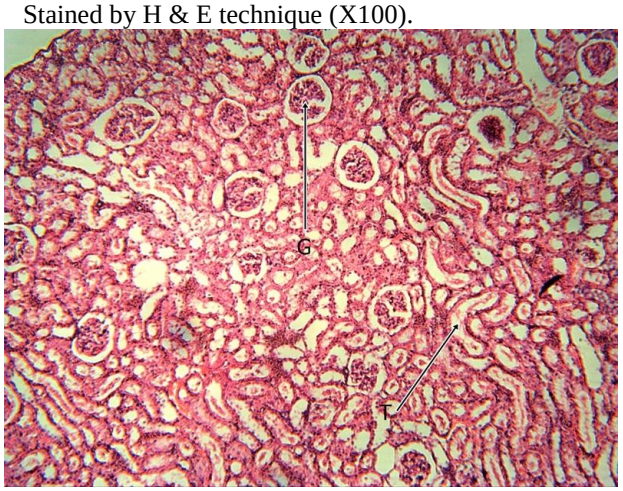

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Plate 3: 1500mg of Goko cleanser Herbal Mixture

Photomicrograph above shows glomeruli that are evenly distributed, of similar size with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. There is mild to moderate haemorrhage into the interstitium and oedema.

Stained by H & E technique (X100).

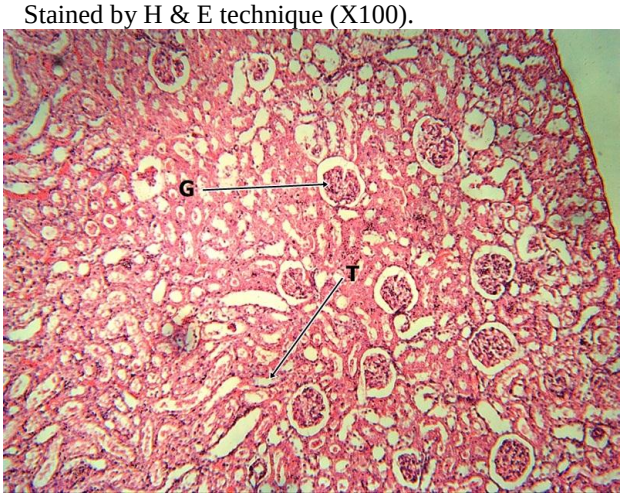

Plate 4: 2000mg of Goko Cleanser Herbal Mixture

Photomicrograph above shows glomeruli that are evenly distributed, of similar size, with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. There is mild to moderate haemorrhage into the interstitium.

Stained by H & E technique (X100)

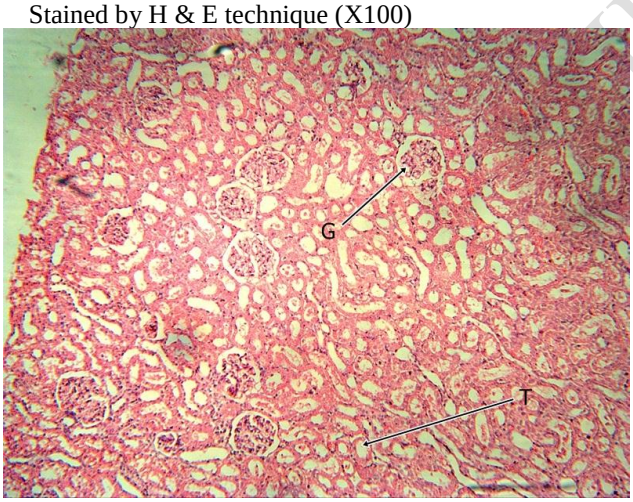

Plate 5: 4000mg of Goko Cleanser Herbal Mixture

Photomicrograph above shows glomeruli that are evenly distributed, of similar size, with normal mesangial cellularity. There are numerous open glomerular capillaries, and normal endothelium. The tubules are of normal density and tubular epithelium is viable. There is mild to moderate haemorrhage into the interstitium and oedema.

Stained by H & E technique (X100)

V. DISCUSSION

Medicinal plants are of great importance to the health of individuals and communities. The medicinal values of these plants lie in some of the phytochemical constituents that produce definite physiological actions in humans. The most important of these bioactive compounds of these plants are alkaloids, tannins, flavonoids and phenolic compounds (Hill, 1952). Many of these constituents are being used by man as spices and food plants, and also possess medicinal values for pregnant and nursing mothers (Okwu, 1999; 2001). Goko cleanser is a poly-herbal mixture and thus is not an exception to having both hepatoprotective and hepatotoxicity roles.

The phytochemical screening of qualitative and quantitative estimation of the percentage of the crude yield of the chemical constituents of Goko cleanser was examined which shows that the herbal mixture was rich in saponins, tannins and flavonoids. However, the quantitative biochemical analysis showed that saponins contain about 7.35%, alkaloids about 0.2% and flavonoids about 4% of the herbal mixture. The saponins, flavonoids, and tannin have shown to possess both hepatoprotective and hepatotoxic properties. This result corresponds with studies by Akinjogunla *et al.,* (2011) and Udochukwu *et al.,* (2015), which noted the presence of Saponin, tannin and flavonoids in aqueous leaf extract of *Vernonia amygdalina;* also a study conducted by Huzaifa *et al*., (2014) and Gazuwa *et al*., (2013) which noted that aqueous extract of *Allium sativa* possess these phyto-chemicals (tannins, saponins and flavonoids); and a study by Amandeep *et al*., (2015) and Feng *et al*., (2014) which noted that *Saccharum officinarum* possess high content of Flavonoids.

The result from this study shows that Goko cleanser herbal mixture cause a significant decrease in body weight and food intake, this could be as a result of the presence of some chemical constituent which can cause inhibitory effects on the appetite centre, thereby causing a decrease in food intake.

Histopathological findings revealed that there was mild to moderate haemorrhage seen in the interstitium of the Kidney. The possible mechanism of action could be as a result of the herbal mixture's phytochemical constituents which causes a break in the membrane of cellular structure of the kidney, and thus leading to increased lipid peroxidation. This further causes an increase in kidney enzymes which serves as biomarkers for organ damage.

VI. CONCLUSION

Findings from this study show that Goko Cleanser herbal mixture possesses dose dependent toxic (haemorrhagic) effect on the kidney, and also cause a decrease in body weight and food intake.

VII. RECOMMENDATION

From this study, we may proceed to recommend that the intake of Goko Cleanser should be minimized as it has no specific dosage. Also further research needs to be done to determine a particular dose that will be less toxic on the kidney. This study also recommends that there is need for further studies on the phytochemical constituents of this herbal mixture so as to determine what causes the increased lipid

peroxidation in the kidney. In addition, patients who seek to take this herbal mixture (Goko Cleanser) should endeavour to always visit the doctor each time they have health issues.

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