Study on the biochemical and histopathological effects of herbal mixture on the kidneys of experimental rats


Abstract

Medicinal plants have shown to possess some biologically active constituents that have significant effects in human. This study was carried out to evaluate the effect of Goko Cleanser (herbal mixture) on the kidney of albino Wistar rats. Phytochemical analysis of the herbal mixture was done. A total of twenty-five animals weighing 150 g – 200 g were used. The rats were randomly selected into five groups of five (5) experimental rats. The groups were classified as A - E, and graded dosage of the herbal mixture administration was done. Group A served as normal control group and received no herbal mixture, group B received 1000 mg/kg of the herbal mixture, group C received 2000 mg/kg of the herbal mixture, group D received 3000 mg/kg and group E received 4000 mg/kg. The results show that Goko Cleanser herbal mixture contains tannin, saponin and flavonoids as its phytochemical constituents. The creatinine level had a significant (P<0.05) increase in test groups D and E when compared to the control group; while in groups B and C, there was no significant increase (P>0.05) when compared to the control group. The urea and potassium levels also had significant increases (P>0.05) in the test groups D and E when compared to the control group. Results of histopathological analysis showed a concomitance with the biochemical findings. Hence, the results from this study show that Goko Cleanser herbal mixture possesses dose-dependent toxic effect on the kidney.
Keywords: Herbal mixture; Vernonia amygdalina; Cajanus cajan; Zingiber officinale; Allium sativum; Saccharum officinarum

1 Introduction

Herbal mixtures have been the strength of traditional medicine for years now. In recent times, some authors have conducted several researches on herbal mixtures or plant extracts to determine its therapeutic effects [1-4]. 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 [5]. Goko cleanser is an 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 an 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 [2, 4]. 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 glycaemic profile of the aqueous extract of Cajanus cajan leaves significantly increases the fasting blood glucose levels of normal rats [6]. 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 an 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 [7]. 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 [8].

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 [3, 9].

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 [10, 11]. 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 biochemical and histopathological effects of this herbal mixture (Goko Cleanser) on the kidney using Wistar rats as experimental models. The aim of this study is to investigate the specific biochemical and histopathological effects of this herbal mixture (Goko Cleanser) on the kidney using Wistar rats.

2 Material and methods

2.1 Materials

Twenty-five (25) albino 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.
2.1.1 **Experimental animals**

The experimental animals were twenty-five albino Wistar rats weighing between 150-200g. 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 four groups, with group 1, 2 and 3 used as the test groups while Group 4 served as the normal 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).

2.1.2 **Collection and identification of the herbal mixture**

The herbal mixture (Goko cleanser) was purchased from a pharmacy shop around Old University of Nigeria Teaching Hospital, Enugu. Phytochemical analysis was carried out to determine the components of the herbal mixture at the Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria, Nsukka Campus.

2.2 **Phytochemical analysis of the herbal mixture (Goko cleanser)**

Preliminary phytochemical screening of the herbal mixture (Goko cleanser) for the presence of glycosides, flavonoids, saponins, steroids, tannins, carbohydrates, proteins and terpenoids was carried out at Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Nigeria Nsukka. Methods according to Trease and Evans [12] were used for the analyses.

2.3 **Acute toxicity test goko cleanser (LD50)**

This was performed on rats and the Lorke procedure of LD50 determination was used [13].

2.4 **Preparation of stock solution**

Two hundred (200ml) of Goko Cleanser was oven dried at 50 °C 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.

\[ 1 \text{ g} = 1000 \text{ mg} \]
\[ 5 \text{ g} = 5000 \text{ mg} = 5000 \text{ mg} / 100 \text{ ml} \]

Stock Solution =50 mg/ml

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

2.5 **Drug administration**

The herbal 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.

2.6 **Experimental protocol**

The rats were then divided into five experimental groups according to their body weight from highest to lowest with five in each group labelled Group A-E. The experimental groups B-E received different doses of drug as follows:

- **Group A** – Did not received the herbal mixture and therefore served as normal control
- **Group B** – Received 1000 mg/kg of Goko cleanser (herbal mixture) daily for 30 days.
- **Group C** – Received 2000 mg/kg of Goko cleanser (herbal mixture) daily for 30 days.
- **Group D** – Received 3000 mg/kg of Goko cleanser (herbal mixture) daily for 30 days.
- **Group E** – Received 4000 mg/kg of Goko cleanser (herbal mixture) daily for 30 days.
2.7 Collection of blood samples
The experimental animals were anaesthetized by chloroform inhalation, followed by cervical dislocation and 2.0ml of blood were collected from the animals through ocular puncture and were placed in a plain tube renal biochemical analysis.

2.8 Biochemical analysis
The levels of Serum Electrolyte, Urea and Creatinine were estimated using the following methods: K+ and Na+ determined using Perlong Medical PL1000A Electrolyte Analyser; Serum urea concentration was determined using the diacetylmonoxime method with protein precipitation according to Natelson et al. [14], Serum creatinine concentration determined using the Jaffe Reaction according to Fabiny and Ertingshausen [15].

2.9 Histopathological analysis
The excised kidneys were processed using the paraffin wax embedding technique, sectioned at 5 microns and stained using the Haematoxylin and Eosin [H and E] staining procedure [16]. The histological sections were examined using an Olympus TM light microscope.

2.10 Statistical analysis
Data analysis was done using GraphPad prism version 7.0 (GraphPad, San Diego, CA, USA). The results of the biochemical assays were reported as mean±SEM (standard error of mean). The level of significance was tested using one-way analysis of variance (ANOVA), followed by the Tukey post hoc analysis. Probability levels less than 0.05 (p<0.05) were considered significant.

3 Results and discussion

3.1 Acute toxicity studies
LD50 value of the herbal extract was 4.5 g/kg which indicates that Goko Cleanser is safe and is not toxic to mice.

3.2 Phytochemical result
The result of the preliminary phytochemical analysis of Goko Cleanser herbal mixture revealed a moderate presence of saponin (++); the trace presence of flavonoids and tannins (+). However, glycosides, alkaloids, proteins and steroids were absent (Table 1).

### Table 1 Qualitative phytochemical results of Goko Cleanser herbal mixture

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Goko Cleanser herbal mixture</th>
<th>Interpretation</th>
</tr>
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<tbody>
<tr>
<td>Saponin</td>
<td>++</td>
<td>Moderately present</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>Present in trace</td>
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<tr>
<td>Tannin</td>
<td>+</td>
<td>Present in trace</td>
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<tr>
<td>Alkaloid</td>
<td>-</td>
<td>Absent</td>
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<tr>
<td>Steroid</td>
<td>-</td>
<td>Absent</td>
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<tr>
<td>Cardiac glycoside</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>Protein</td>
<td>-</td>
<td>Absent</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>-</td>
<td>Absent</td>
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</tbody>
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3.3 Biochemical results
The functionality of the kidneys was established by estimating the serum level of the kidney biochemical markers; urea, creatinine, potassium (K+) and sodium (Na+) (Figures 1 and 4) The oral administration of Goko Cleanser herbal mixture caused a steady rise in serum urea, creatinine and K+ levels in a dose-dependent manner. There was a significant increase in the serum urea levels of rats that received 4000mg/kg body weight of the herbal mixture when compared...
with the normal control rats (p<0.05). Again, there were significant increases in the serum urea levels of rats that received 3000mg/kg and 4000mg/kg body weight of the herbal mixture separately, when compared with the normal control rats (p<0.05). Furthermore, oral administration of Goko Cleanser herbal mixture caused a steady decrease in serum sodium levels in a dose-dependent manner. There was a significant decrease in the serum sodium levels of rats that received 4000mg/kg body weight of the herbal mixture when compared with the normal control rats (p<0.05).

**Figure 1** Effect of graded doses of Goko Cleanser herbal mixture on serum urea levels

Histogram show the serum urea levels of rats in the experimental groups. The preliminary data show oral administration of Goko Cleanser herbal mixture caused a steady rise in serum urea levels in a dose-dependent manner. There was a significant increase in the serum urea levels of rats that received 4000mg/kg body weight of the herbal mixture when compared with the normal control rats (p<0.05). The data are presented as mean±SEM of serum urea (mmol/L) for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using ANOVA (*p˂0.05); n.s implies no significant difference (p>0.05).

**Figure 2** Effect of graded doses of Goko Cleanser herbal mixture on serum creatinine levels
Histogram show the serum creatinine levels of rats in the experimental groups. The preliminary data show oral administration of Goko Cleanser herbal mixture caused a steady rise in serum creatinine levels in a dose-dependent manner. There were significant increases in the serum urea levels of rats that received 3000mg/kg and 4000mg/kg body weight of the herbal mixture separately, when compared with the normal control rats (p<0.05). The data are presented as mean±SEM of serum creatinine (µmol/L) for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using ANOVA (*p<0.05); n.s implies no significant difference (p>0.05).

**Figure 3** Effect of graded doses of Goko Cleanser herbal mixture on serum potassium levels

Histogram show the serum potassium levels of rats in the experimental groups. The preliminary data show oral administration of Goko Cleanser herbal mixture caused a steady rise in serum potassium levels in a dose-dependent manner. There was a significant increase in the serum potassium levels of rats that received 4000mg/kg body weight of the herbal mixture when compared with the normal control rats (p<0.05). The data are presented as mean±SEM of serum urea (mmol/L) for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using ANOVA (*p<0.05); n.s implies no significant difference (p>0.05).

**Figure 4** Effect of graded doses of Goko Cleanser herbal mixture on serum sodium levels
Histogram show the serum sodium levels of rats in the experimental groups. The preliminary data show oral administration of Goko Cleanser herbal mixture caused a steady decrease in serum sodium levels in a dose-dependent manner. There was a significant decrease in the serum sodium levels of rats that received 4000mg/kg body weight of the herbal mixture when compared with the normal control rats (p<0.05). The data are presented as mean±SEM of serum urea (mmol/L) for individual treatment. See Materials and Methods for experimental details. Statistical analyses were performed using ANOVA (*p˂0.05); n.s implies no significant difference (p>0.05).

3.4 Histopathological results.
The kidney nephrons of normal control rats (Group A) appeared structurally and functionally normal. The nephrons showed a well conserved morphology; the glomeruli and tubules appear normal. In the kidney section of rat administered with 1000mg/kg Goko cleanser (group B), the glomeruli and the tubules appear normal. Interestingly, as the dose of the herbal mixture increased, the glomeruli of rats administered with 2000mg/kg Goko cleanser (group C), 3000mg/kg Goko cleanser (group D) and 4000mg/kg Goko cleanser (group B), showed increased distortion of histoarchitecture of the kidney section, depicting increasing injury in the rat kidney.

Figure 5 Photomicrograph of kidney section from normal control rat (group A). Features: The glomeruli (G) and the tubules (T) appear normal. Stain: Haematoxylin and eosin. Magnification: X100

Figure 6 Photomicrograph of kidney section from rat administered with 1000mg/kg Goko cleanser (group B). Features: The glomeruli (G) and the tubules (T) appear normal. Stain: Haematoxylin and eosin. Magnification: X100
Figure 7 Photomicrograph of kidney section from rat administered with 2000mg/kg Goko cleanser (group C). Features: A few glomeruli are constricted while some appear normal; the tubules (T) appear normal. Stain: Haematoxylin and eosin. Magnification: X100

Figure 8 Photomicrograph of kidney section from rat administered with 3000mg/kg Goko cleanser (group D). Features: The glomeruli are constricted (G); the tubules (T) appear normal. Stain: Haematoxylin and eosin. Magnification: X100

Figure 9 Photomicrograph of kidney section from rat administered with 4000mg/kg Goko cleanser (group E). Features: A Bowman’s capsule is devoid of glomeruli (*) while others (G) are partly eroded; the tubules (T) appear normal. Stain: Haematoxylin and eosin. Magnification: X100
Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants lies 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 [11]. Many of these constituents are being used by man as spices and food plants and also possess medicinal values for pregnant and nursing mothers [17, 18]. Goko cleanser a poly-herbal mixture which constitutes of several medicinal plants is not an exception; however, its effects are not elucidated. In this present study, the phytochemical screening of qualitative estimation of the bioactive constituents of Goko cleanser was examined which shows that the herbal mixture contains appreciable amounts of saponins, tannin and flavonoids. The saponins, flavonoids, and tannin have shown to possess medical activity as well as exhibiting physiological activity. This result corresponds with studies by Akinjogunla et al. [4] and Udochukwu et al. [2], which noted the presence of Saponin, tannin and flavonoids in aqueous leaf extract of Vernonia amygdalina; also a study conducted by Gazuwa et al. [8] which noted that aqueous extract of Allium sativa possess these phyto-chemicals (tannins, saponins and flavonoids); and a study by Amandeep et al [3] and Feng et al. [9] which noted that Saccharum officinarum possess high content of Flavonoids. Findings from this study revealed there was no significant increase in Urea level in the treated groups except for the highest dose group (4000mg/kg) when compared with normal control rat. However, for Creatinine level, there was a significant increase in the treated groups (3000mg/kg and 4000mg/kg) separately when compared with the control group. The oral administration of Goko Cleanser herbal mixture caused a steady rise in serum urea, creatinine and K+ levels in a dose-dependent manner. Furthermore, oral administration of Goko Cleanser herbal mixture caused a steady decrease in serum sodium levels in a dose-dependent manner. There was a significant decrease in the serum sodium levels of rats that received 4000mg/kg body weight of the herbal mixture when compared with the normal control rats (p<0.05). The possible mechanism of action could result from the toxic substances present in the herbal mixture, thus leading to disruption of the biochemical and physiological actions of the kidney, since an increase in Creatinine and Urea serves as biomarkers for kidney damage. The amount of creatinine is usually constant, so that elevated levels indicate diminished renal function only, since it is easily excreted by the kidneys. This contradicts work done by Atangwho et al. [19] which reported that there was a significant reduction in urea and creatinine level in diabetic rats when treated with ethanolic extract of V.amygdalina.

4 Conclusion

Findings from this present study show that Goko Cleanser herbal mixture possesses toxic effect on the kidney on dose dependent. The results offer scientific evidence that in low doses when used to treat ailments, Goko Cleanser herbal mixture has no harmful effects and could very well be potentially therapeutic or efficacious; however higher doses or prolonged use as a herbal preparations calls for caution in patients with underlying kidney dysfunction.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare they have no conflict of interests.

Statement of ethical approval

The experimental procedure was approved by the institution’s animal ethics committee at the University of Nigeria Teaching Hospital (UNTH/CSA. 902/VOL. 11).

References


