



## Haematological Study on the Effects of Goko Cleanser (Herbal Mixture) on Adult Female Wistar Rats

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### Authors' contributions

This work was carried out in collaboration of all authors. Author DNO designed the study, wrote the protocol, supervised the experimental study and wrote the first draft of the manuscript. Author MWC acquired the animals, conducted the toxicity test and herbal mixture identification. Author MCI carried out the blood analysis and assisted author MWC in the herbal mixture identification. Author OSN assisted author MWC in carrying out the toxicity test. Author OIJ carried out the statistical analysis. Author ECE managed the literature searches. All authors read and approved the final manuscript.

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### ABSTRACT

**Introduction:** Goko cleanser is one of the popular herbal mixtures used by the Nigerian populace. This study was carried out to evaluate the effects of this herbal mixture on some haematological parameters of adult female Wistar rats.

**Methodology:** A total of twenty five Wistar rats weighing about 160 – 280g were divided into five groups according to their corresponding weights. The groups were designated as groups 1 – 5. Group 1 served as the control group, while groups 2, 3, 4, and 5 received 1000mg/Kg, 1500mg/Kg, 2000mg/Kg and 4000mg/Kg respectively.

**Results:** The TWBC, PCV, PLATELET count, and RBC and Hb showed a statistically significant ( $p < 0.05$ ) increase for the test groups 2 – 5 when compared to the control group. The body weight

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had a statistically significant ( $p < 0.05$ ) decrease in groups 2, 4, and 5; while group 3 had a statistically insignificant ( $p > 0.05$ ) decrease.

**Conclusion and Recommendation:** This study revealed that Goko Cleanser contains some haemopoietic benefits, but inhibit appetite centres. Hence, this study recommends that further study be carried out on the effects of this herbal mixture on the brain so as to reveal the extent of its effect on the appetite centres.

**Keywords:** Herbal mixtures; haematological parameters; blood analyses; body-weight analysis.

## 1. INTRODUCTION

Herbal medicine is renowned as the most common form of alternative medicine and is used by about 60% of the world population both in the developing countries and in the developed countries where modern medicines are predominantly used ([1]; [2]). In rural communities, the exclusive use of herbal drugs, prepared and dispensed by herbalists without formal training, for the treatment of diseases is still very common [2]. Thus, herbal medicines have received greater attention as alternative to clinical therapy in recent times leading to subsequent increase in their demand [3]. Mixtures of these herbs may contain a whole plant, parts of a plant or extract of either one or a combination of plants. Many of these mixtures (for example Goko cleanser) are commonly sold today, without considering their associated potential toxicities.

Although plants generally contain bioactive principles believed to be responsible for their therapeutic effects, they also contain some phytotoxins and other heavy metals contaminants whose toxicological actions have always been ignored [4]. Goko cleanser mixture is a combination of herbs such as *Vernonia amygdalina*, *Saccharum officinarum*, *Allium sativum*, *Cajanus cajan*, Caramel, and *Zingiber officinale*.

*Vernonia amygdalina*, bitter leaf (locally called onugbu in Igbo) is a member of the squash family of plants. They are consumed as cooked complements to major staple foods such as cassava, pounded yam, guinea corn, maize, millet, rice and plantain. It is of a high medicinal value as the juice extracted from the leaves are wholly applied to fresh wounds or cuts in some rural community [5].

*Saccharum officinarum* (sugar cane, and locally referred to as Okpete in Igbo, Ireke in Yoruba, and Rake in Hausa) is a large strong-growing species of grass in the genu – *Saccharum*. This plant is grown in warm temperate or tropical

regions. It is a fibrous stalk which is about 2m – 6m tall and its juice contains high quantity of sugar [6] used for the production of sugar and other products.

*Allium sativum*, garlic is a member of the *Allium* family and is among the first cultivated crops in the world due to their long storage time and portability ([7]; [8]). Garlic is best known as a wonderful herb that promotes plenty of benefits towards health and has medicinal properties. It possesses antibacterial and antifungal activities, and contains powerful sulphur and other numerous phenolic compounds which arouse great interest [7].

*Zingiber Officinale* commonly called ginger belongs to the family Zingiberaceae. The plant is a knotted, thick, and beige underground stem (rhizome). Today, ginger root is widely used as a digestive aid for mild stomach upset and is commonly recommended by health care professionals to help prevent or treat nausea and vomiting associated with motion sickness, pregnancy, and cancer chemotherapy (Bone et al., 1990; [9]; [10]). Ginger is used as support in inflammatory conditions such as arthritis [11], and may even be used in heart disease [12] or cancer [13].

*Cajanus cajan* commonly called pigeon pea is a perennial legume from the family Fabaceae, which is grown primarily for their seed. Their seed is highly nutritious and consumed largely for their sources of protein, carbohydrates, vitamins and minerals ([14]; [15]). It contains biologically active substances such as saponins, sterols, glycosides, tannins and flavanoids [16]. Some plant extracts with these chemicals have protective effects on the body organs [17].

Haematological parameters are the combination of various physiological parameters such as the erythrocyte count (RBC), white blood cell count (WBC), packed cell volume (PCV), platelet count, haemoglobin (Hb), e.t.c. These parameters are very essential for life, and imbalance in any of these indices can lead to various physiological

disorders. Haematological parameters convey the functional status of blood [18]. Therefore, this study is aimed at establishing the roles of Goko Cleanser herbal mixture on these blood parameters.

## 2. METHODOLOGY

### 2.1 Materials

The materials used to carry out this experimental study include twenty five female Wistar rats, Goko Cleanser Herbal Mixture, plastic cages with iron netting, oral cannula, saw dust; sets of EDTA treated sample bottles, distilled water, latex gloves, animal weighing balance (CAMRY LB11), electronic weighing balance (NAPCO PRECISION J410), Growers Mesh, 10ml hypodermic syringe, dissecting kits, and cotton wool.

### 2.2 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.

### 2.3 Experimental Animals

Twenty five female albino Wistar rats weighing 160-280g were procured from the animal farm of the department of Anatomy, Nnamdi Azikiwe University, Nnewi campus. 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. The rats were divided into five groups of five animals each. The test groups include groups 1, 2, 3, and 4 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 LB11).

### 2.4 Collection and Identification of the Herbal Mixture

The herbal mixture (Goko Cleanser) was purchased from a NAFDAC (National Agency for Food and Drugs Administration and Control) and Pharmaceutical council certified pharmacy store in Nnewi, Nigeria. This herbal mixture constitute a mixture of the following herbs: *Vernonia amygdalina*, *Saccharum officinarum*, *Allium sativum*, *Cajanus cajan*, Caramel, and *Zingiber officinale*.

## 2.5 Toxicity Test of Goko Cleanser (Calculation of LD50)

The LD50 was performed according to the method described by Lorke's in 1983. The method involved two phases and the LD50 was represented as thus:

$D_0$  = Highest dose that gave no mortality,  
 $D_{100}$  = Lowest dose that produced mortality.

### Phase 1

This phase required nine animals. The nine animals were divided into three groups of three animals each. Each group was administered different doses of the test substance.

GROUP 1 - Received 10mg/kg of the drug

GROUP 2 - Received 100mg/kg of the drug

GROUP 3 - Received 1000mg/kg of the drug

The animals were placed under observation for 24 hours so as to monitor the behavioural changes and anato-physiological conditions. After five days, there were no casualties (mortalities).

### Phase 2

This phase involved the use of three animals which were distributed into three groups of one animal each. The animals were administered higher doses of the test substance and then observed for 24 hours for behavioural changes as well as mortality.

GROUP 1 Received 2600mg/kg

GROUP 2 Received 3900mg/kg

GROUP 3 Received 5000mg/kg

The group that received 5000mg/kg died within 24hours whereas the group that received 3900mg/kg died after 24hrs.

The formula below was used to calculate the LD50

$$LD50 = \sqrt{a \times b}$$

Where a = maximum dose with 0% mortality (2600 mg/kg)

b = minimum dose with 100% mortality (3900 mg/kg)

$$LD50 \text{ of Goko Cleanser} = \sqrt{2600 \times 3900} = 3184.34 \text{ mg/kg}$$

LD50 of the Goko Cleanser Herbal Mixture of Female Wistar Rats for this study is 3184.34 mg/kg. This is the median lethal dose of Goko cleanser herbal mixture.

**Table 1.1. Visible Observation for LD50 calculation during Phase 1**

Groups	Death rate	Visible observation
1	0/3	They were normal.
2	3/3	Weak and died after 24hrs
3	3/3	Very weak and died within 24hrs

## 2.6 Preparation of Stock Solution

200ml of Goko Cleanser was oven dried at 50°C and the concentrated Goko Cleanser was measured to be 5g  
 5g = 5000mg  
 5g of Goko cleanser was dissolved in 100mls of distilled water to get a stock solution 50mg/ml.

### 2.6.1 Stock solution

$$1g = 1000mg$$

$$5g = 5000mg = 5000mg/100ml$$

Therefore, the stock solution is 50mg/ml.

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

## 2.7 Drug Administration

The herbal mixture was administered to the rats in the test groups 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 groups were allowed to feed and drink water *ad libitum*.

## 2.8 Experimental Protocol

Animals were first weighed before the administration of the herbal mixture. Then, animals were divided into five experimental groups according to their body weight from highest to lowest with five in each group labelled Groups 1 – 5. The experimental groups received different doses of drugs.

**Table 1.2. Dosage of herbal mixture administered to animals**

Groups	Dosage (ML)
1	Nil
2	4.16
3	6.00
4	8.96
5	18.56

## 2.9 Collection of Blood Samples

2.0 ml of blood was collected from the animals through ocular puncture and were placed in lithium heparin bottles for hormonal assay (FSH and oestrogen) and EDTA tubes for blood test.

### 2.9.1 Precautions

1. This study ensured that sterilized instruments were used to carry out the experiment.
2. The study ensured that equal volumes of blood were collected from the rats.
3. This study ensured that blood samples used for hormonal assay were put in lithium heparin bottles prior to analysis.
4. This study ensured that different bottles were used for different blood samples.
5. This study ensured that each sample bottles were properly labelled.

## 2.10 Data Analysis

Data were analyzed using Statistical Package for Social Sciences (SPSS) and values expressed as mean ± standard error of mean (S.E.M) using one way analysis of variance (ANOVA), followed by post Hoc LSD. Results were considered significant at  $p \leq 0.05$ .

## 3. RESULTS

All data were analyzed using one-way ANOVA and data were considered significant at  $P \leq 0.05$ . Values were represented as mean and SEM. The result above reveals that the Total White Blood Cell (TWBC) showed a statistically significant increase across all treated groups; 2 (9.00±0.04), 3 (6.87±0.04), 4 (8.37±0.07), and 5 (8.77±0.17), as compared with the control group (5.42±0.04). The PCV analysis showed a statistically significant increase in the treated groups; 2 (0.37±0.01), 3 (0.35±0.00), 4 (0.40±0.00), and 5 (0.44±0.00), when compared with the control group (0.30±0.01).

All data were analyzed using one-way ANOVA and considered significant at  $P \leq 0.05$ . Values were represented as mean and SEM. Result

above shows that, the Platelet count increases across all treated groups 2 (700.75±1.25), 3 (710.75±1.37), 4 (817.25±4.21), and 5 (841.75±3.14) when compared with the control group (625.00±10.94), and the increase in platelet count was statistically significant in all treated groups. The result on RBC shows a statistically significant increase in treated groups 2 (6.05±0.10), 3 (6.10±0.10), 4 (6.48±0.00), and 5 (6.83±0.08) when compared with the control group (5.41±0.13). The Haemoglobin result shows a statistically significant increase across all treated groups 2 (12.37±0.14), 3 (11.65±0.13), 4 (12.90±0.04) and 5 (14.32±0.04) when compared with the control group (8.97±0.08).

All data were analyzed using one-way ANOVA and values were considered significant at P≤0.05. Values were represented as mean and SEM. Results from the table above revealed that there was an increase in food intake of the animals in day 7 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 with the control group (28.50±2.98). However, this increase in food intake was not statistically significant. In day 14, there was a decrease in food intake in all test groups 2 (32.07±2.02), 3 (29.12±1.21), 4 (20.25±0.32) and 5 (14.25±1.63), when compared with the control group (46.50±3.88), but the decrease in food intake was statistically significant in groups 4 and 5. In day 21, there was a statistically significant decrease in food intake in groups 2 (24.50±0.64), 3 (23.87±0.59), 4 (20.25±0.85) and 5 (13.75±1.65) when compared with the control group (46.50±3.88).

All values were analyzed using dependent t-test and values were considered significant at P≤0.05. Results from the table above shows that there was a statistically significant increase in the body weight of animals in the control group. In groups 2, 3 and 5, there was a statistically significant decrease in the body weight; while in group 3, there was a statistically insignificant decrease in the body weight.

**Table 2.1. Effect of Goko Cleanser (herbal mixture) on TBC and PCV after 3 weeks of treatment**

		Mean	±Sem	P-value
<b>Total WBC (10<sup>3</sup>/μL)</b>	GROUP 1 (Control)	5.42	±0.04	
	GROUP 2 (1000mg)	9.00	±0.04	0.000
	GROUP 3 (1500mg)	6.87	±0.04	0.000
	GROUP 4 (2000mg)	8.37	±0.07	0.000
	GROUP 5 (4000mg)	8.77	±0.17	0.000
<b>Pack Cell Volume (μL)</b>	GROUP 1 (Control)	0.30	±0.01	
	GROUP 2 (1000mg)	0.37	±0.01	0.000
	GROUP 3 (1500mg)	0.35	±0.00	0.000
	GROUP 4 (2000mg)	0.40	±0.00	0.000
	GROUP 5 (4000mg)	0.44	±0.00	0.000

**Table 2.2. Effect of Goko Cleanser herbal mixture on Platelet Count, RBC, and Hb after 3 weeks of treatment**

		Mean	±Sem	P-value
<b>Platelet Count (X 10<sup>3</sup>/μL)</b>	Group 1 (Control)	625.00	±10.94	
	Group 2 (1000mg)	700.75	±1.25	0.000
	Group 3 (1500mg)	710.75	±1.37	0.000
	Group 4 (2000mg)	817.25	±4.21	0.000
	Group 5 (4000mg)	841.75	±3.14	0.000
<b>Red Blood Cell (X 10<sup>12</sup>/L)</b>	Group 1(Control)	5.41	±0.13	
	Group 2 (1000mg)	6.05	±0.10	0.000
	Group 3 (1500mg)	6.10	±0.10	0.000
	Group 4 (2000mg)	6.48	±0.00	0.000
	Group 5 (4000mg)	6.83	±0.08	0.000
<b>Haemoglobin (g/dL)</b>	Group 1 (Control)	8.97	±0.08	
	Group 2 (1000mg)	12.37	±0.14	0.000
	Group 3 (1500mg)	11.65	±0.13	0.000
	Group 4 (2000mg)	12.90	±0.04	0.000
	Group 5 (4000mg)	14.32	±0.04	0.000

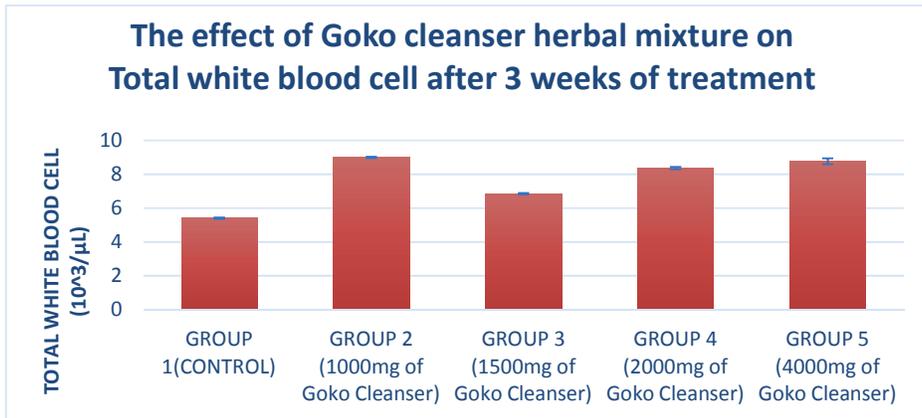


Fig.1.1. Bar chart showing the effect of Goko Cleanser herbal mixture on Total white blood cell level after 3 weeks of treatment

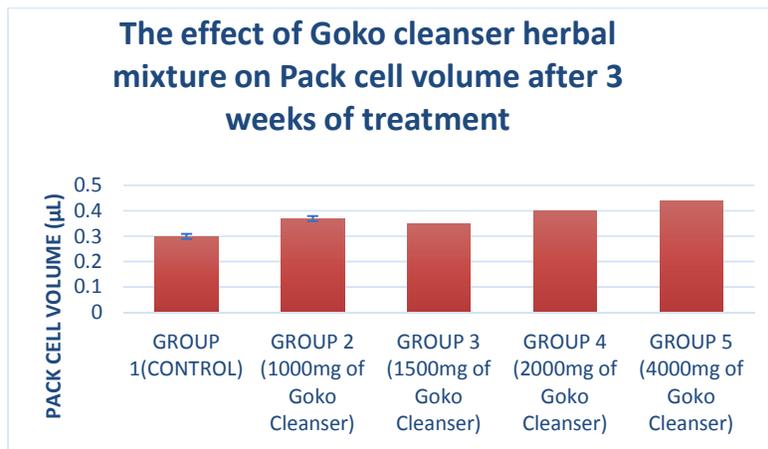


Fig. 1.2. Bar chart showing the effect of Goko Cleanser herbal mixture on Pack Cell Volume level after 3 weeks of treatment

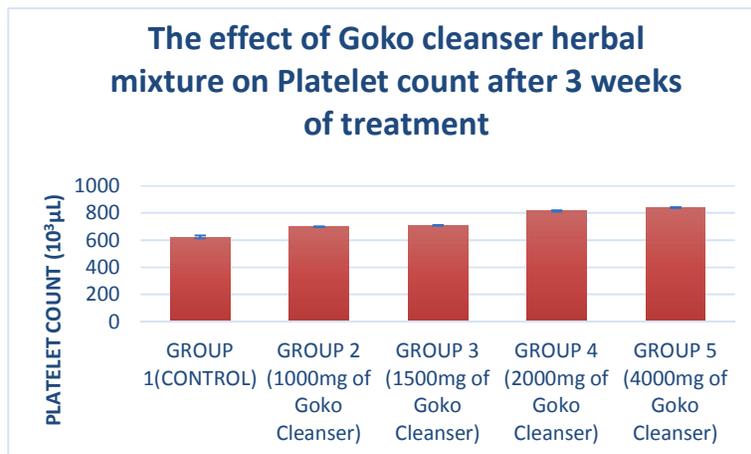


Fig. 2.1. Bar chart showing the effect of Goko cleanser herbal mixture on Platelet Count Level after 21 days of treatment

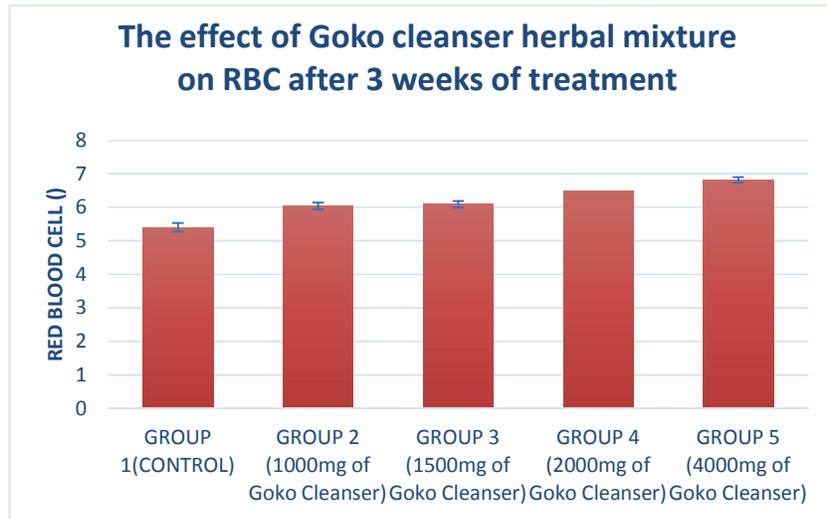


Fig 2.2. Bar chart showing the effect of Goko cleanser herbal mixture on Red Blood Cell Level after 3 weeks of treatment

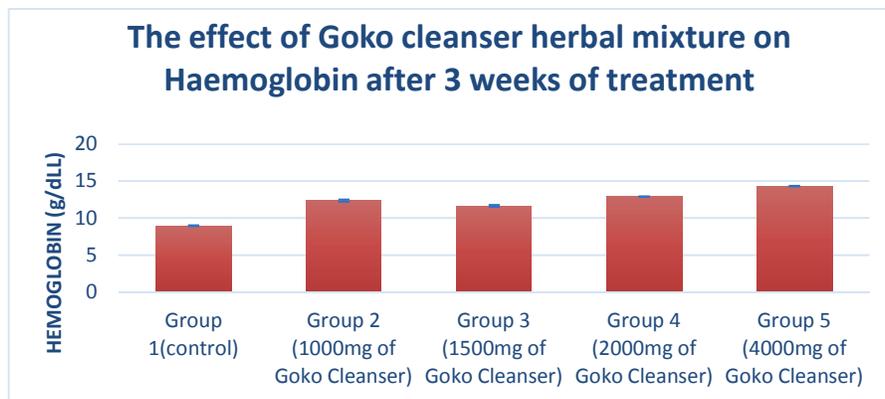


Fig. 2.3. Bar chart showing the effect of Goko Cleanser herbal mixture on Haemoglobin level after 3 weeks of treatment

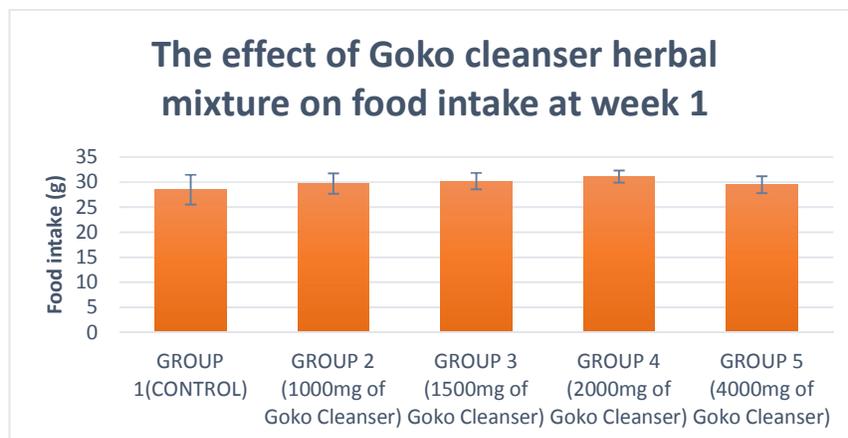
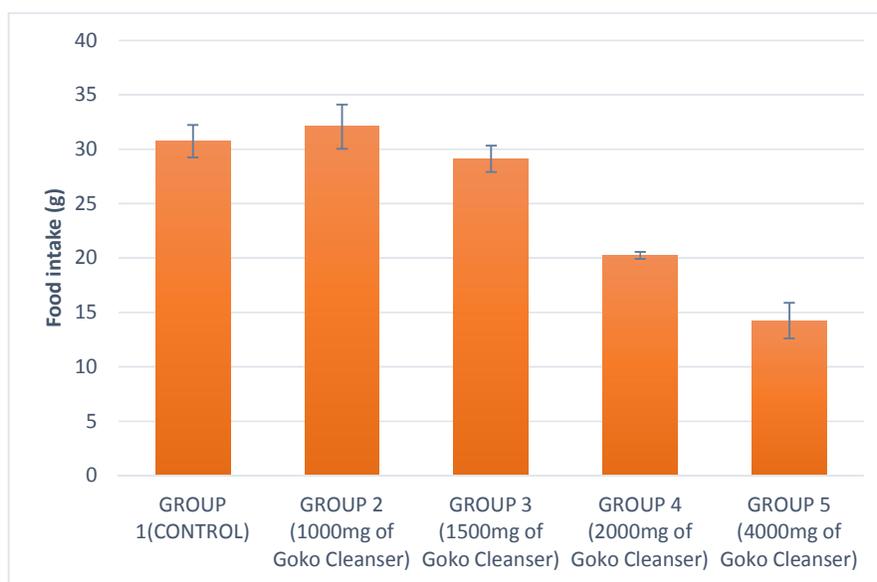


Fig. 3.1. Bar chart showing the Effect of Goko Cleanser herbal mixture on food intake at week 1

**Table 2.3. Effect of Goko Cleanser herbal mixture on Food Intake after three weeks of treatment**

Food intake		Mean	±Sem	P-value	F-value
<b>Week 1</b>	GROUP 1 (Control)	28.50	±2.98	-	0.232
	GROUP 2 (1000mg)	29.72	±2.03	0.671	
	GROUP 3 (1500mg)	30.22	±1.64	0.551	
	GROUP 4 (2000mg)	31.12	±1.21	0.368	
	GROUP 5 (4000mg)	29.52	±1.66	0.722	
<b>Week 2</b>	GROUP 1 (Control)	30.75	±1.49	-	28.130
	GROUP 2 (1000mg)	32.07	±2.02	0.529	
	GROUP 3 (1500mg)	29.12	±1.21	0.442	
	GROUP 4 (2000mg)	20.25	±0.32	0.000	
	GROUP 5 (4000mg)	14.25	±1.63	0.000	
<b>Week 3</b>	GROUP 1 (Control)	46.50	±3.88	-	38.401
	GROUP 2 (1000mg)	24.50	±0.64	0.000	
	GROUP 3 (1500mg)	23.87	±0.59	0.000	
	GROUP 4 (2000mg)	20.25	±0.85	0.000	
	GROUP 5 (4000mg)	13.75	±1.65	0.000	



**Fig. 3.2. Bar chart showing the Effect of Goko cleanser herbal mixture on food intake at week 2**

**Table 2.4. Effect of Goko Cleanser herbal mixture on the Initial and Final body weight after 3 Weeks of treatment**

		Mean	±Sem	P-value	T-Value
<b>Group 1 (CONTROL)</b>	Initial	200.00	±16.32	0.035	-3.656
	Final	235.00	±9.57		
<b>Group 2 (1000mg)</b>	Initial	210.00	±25.16	0.049	3.220
	Final	182.50	±17.01		
<b>Group 3 (1500mg)</b>	Initial	192.50	±12.50	0.069	2.777
	Final	162.50	±4.78		
<b>Group 4 (2000mg)</b>	Initial	220.00	±11.54	0.007	6.734
	Final	162.50	±6.29		
<b>Group 5 (4000mg)</b>	Initial	255.00	±22.17	0.025	4.196
	Final	147.50	±4.78		

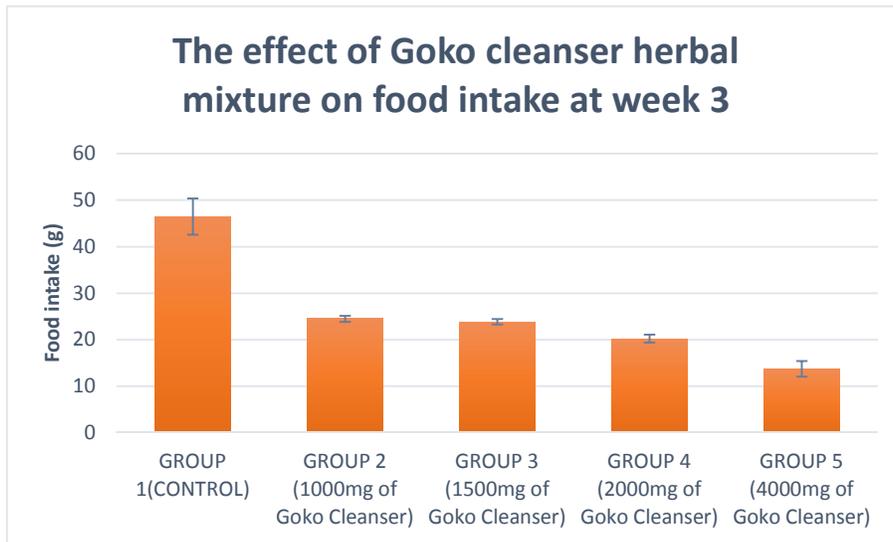


Fig. 3.3. Bar chart showing the Effect of Goko cleanser herbal mixture on food intake at week 3

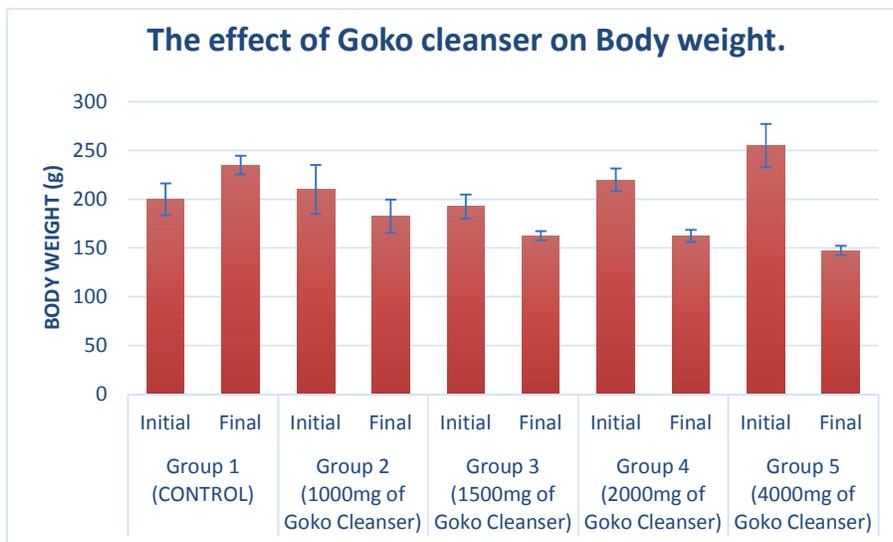


Fig. 4.1. Bar chart showing the effect of Goko Cleanser herbal mixture on body weight at 3 weeks

#### 4. DISCUSSION

Treatment of ailments using medicinal plants has a long age practice in Africa and especially Nigeria. Recent study shows that 80% of the world population consume medicinal plants, out of which 30% of this population was prescribed by physicians [19]. In addition to this, it was estimated by the WHO that 80% of the population of developing countries believe in traditional medicine (which consists of herbal

mixtures used in 85% of the cases) for primary health assistance [20]. The increasing popularity in the use of herbal medicine produced by traditional practitioners, especially among the rural populace in developing countries as source of medical care could be attributed to its advantages of being readily available and cheap.

According to Onyejike et al [21] the phytochemical screening of Goko Cleanser

herbal mixture reveals that saponin, tannin and flavonoids were present in 7.35%, 0.2% and 4% respectively. Alkaloid, steroid, tapernoid, cardiac glycoside, protein, and carbohydrate were absent. These phytochemicals present in this herbal mixture possess medicinal value ([21]; [22]). Findings from the study carried out on all the blood parameters after a three-week intake of Goko Cleanser herbal mixture revealed that there was a statistically significant increase of TWBC, PCV, Platelet count, RBC, and Hb in all treated groups when compared with the control. This contradicts a study carried out by Eyo et al. [23] on the effects of aqueous root extracts of *Vernonia amygdalina* on *Rattus norvegicus* which presented a statistical insignificance. This suggests that a mixture of the constituents (phytochemicals) of Goko Cleanser suppresses the effect of *Vernonia amygdalina*, thereby presenting a haemopoietic benefit. However, findings from the studies on TWBC, PCV, Platelet count, RBC, and Hb correlates with that of Olaniyan et al. [24] whose study on the effects of aqueous extract of garlic (*Allium sativum*) on blood parameters in adult Wistar rats presented a statistically significant increase on the RBC, TWBC, and PCV; and also correlate with a study carried out by Sweeney et al. [25] who reported that *Vernonia amygdalina* enhances the immune system.

Results from animal food intake rate and body weight after a three-week treatment with Goko Cleanser herbal mixture reveals a statistically significant decrease in food intake and body weight. This suggests that some of the phytochemical constituents of this herbal mixture cause inhibitory effects on the appetite centres, thereby causing a decrease in food intake and body weight. This corresponds with findings from a study by Augusti et al [26] which noted that prolonged feeding of high levels of raw garlic in rats resulted in anaemic conditions, weight loss and growth impedance due to lysis of red blood cells.

## 5. CONCLUSION

Following the findings from this study, this paper concludes that Goko Cleanser herbal mixture increases haematological parameters (TWBC, PCV, Platelet count, RBC, and Hb) and presents great haematopoietic properties. It can also be deduced from this study that Goko Cleanser herbal mixture possesses anti-anaemic properties. However, this herbal mixture inhibits appetite centres.

## 6. RECOMMENDATIONS

Following the results obtained from this study, this paper will proceed to recommend the following:

1. This herbal mixture is a good source of weight loss therapy for individuals suffering from obesity as it will inhibit appetite centres, reduce body weight, and further increase blood parameters required to keep fit.
2. Intake of this herbal mixture should be done with appropriate dosage prescribed by a physician.
3. Further studies should be carried out on the effects of this herbal mixture on the brain so as to ascertain the extent of its effect on the appetite centres.

## DECLARATION

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## DISCLAIMER

Some part of this manuscript was previously presented and published in the following conference "18th Annual Scientific Conference of Society of Experimental and Clinical Anatomists of Nigeria" At Enugu, Enugu State, Nigeria in November 2019, Web Link of the proceeding: [https://www.researchgate.net/publication/341105466\\_Haematological\\_study\\_on\\_the\\_effects\\_of\\_Goko\\_Cleanser\\_Herbal\\_Mixture\\_on\\_adult\\_femal\\_e\\_Wistar\\_rats](https://www.researchgate.net/publication/341105466_Haematological_study_on_the_effects_of_Goko_Cleanser_Herbal_Mixture_on_adult_femal_e_Wistar_rats)

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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