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Effacts of Goko Herbal Cleanser Mixture on Haematological Parameters in Wistar Rats

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Abstract: **Original Research**

Background: Goko herbal cleanser mixture is a popular herbal preparation used in Nigeria and other African countries for microbial infections and diseases management. Its bio-active compounds influence several biological processes which has also raises some safety concern. The present study is aimed at determining the impact of Goko herbal cleanser mixture on hematological parameters of Wistar rats. ***Methods***: An experimental laboratory design method was adapted in the study, which involved Twelve Wistar rats, grouped into control, medium-dose (0.5ml/kg) and high-dose (1ml/kg) categories, with Goko cleanser herbal mixture administered over 21 days. Hematological parameters were analyzed using hematology analyzer method and data were evaluated statistically. ***Result:*** The data revealed that moderate doses of Goko cleanser herbal mixture improved RBC and platelet counts, potentially supporting erythropoiesis and thrombopoiesis. However, higher doses of Goko cleanser herbal mixture reduces the RBC quality, mean corpuscular volume (MCV), and mean corpuscular hemoglobin (MCH), which suggest potential risk of toxicity. ***Conclusion:*** Goko herbal cleanser mixture elicit a dose-dependent impacts on erythropoiesis and thrombopoiesis, highlighting the need for careful dosage regulation to maximize therapeutic outcome and reduces the adverse effects of high doses.

Keywords: Goko herbal cleanser mixture, Wistar Rat, erythropoiesis Blood tissue, Thrombopoiesis.

INTRODUCTION

Bitter extracts have been utilized in traditional medicine for centuries, renowned for their diverse therapeutic properties. These extracts are typically derived from plants rich in phytochemicals such as alkaloids, flavonoids, saponins, and tannins, which contribute to their medicinal effects [1]. For example, the bitter melon (Momordica charantia) has been extensively studied for its antidiabetic, anti-inflammatory, and antioxidant activities [2]. Similarly, neem (*Azadirachta indica*) [3] and the bitter components of bitter kola [4] are known for their antimicrobial and anti-parasitic properties. Despite the widespread use of bitter extracts, there is a significant gap in the scientific understanding of their effects on hematological parameters.

Previous studies have primarily focused on the therapeutic effects of bitter extracts, such as their antimicrobial, anti-inflammatory, and antioxidant properties [5, 6]. However, the effect of the extracts on blood parameters remains under explored. Understanding these effects is crucial, as changes in hematological parameters can

provide early indications of potential toxicities or therapeutic benefits [7]. Hematological parameters including red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin concentration, and platelet count are critical indicators of the body's health which assess functional characteristics of blood and blood forming organs [8]. These parameters provide valuable insights into the physiological and pathological state of an organism, reflecting its ability to transport oxygen, mount an immune response, and maintain hemostasis [9]. Alterations in these parameters can indicate various health conditions, including anemia, infection, inflammation, and bleeding disorders [10].

Wistar rats are commonly used as a model organism in biomedical research which offer a controlled environment to study the effects of bitter extracts on hematological parameters. Their well-documented genetic and physiological characteristics make them suitable for such investigations [11]. This study aimed at bridging the knowledge gap by experimentally evaluating the impact of Goko bitter extract on the hematological parameters in Wistar rats, thereby providing a scientific basis for the traditional use of these extracts and contributing to the field of phytomedicine.

MATERIALS AND METHOD

This study was designed as a controlled laboratory experiment to assess the effects of Goko cleanser herbal mixture administered to the Wistar rat on hematological parameters. Twelve Wistar rats were caged and maintained under the standard husbandry condition (between 22℃, 12 hours light and 12 hours dark) in the animal house. Animals were fed with chow and water *ad libitum.* The use of experimental animals followed the Animal Ethical Committee of the Bingham University Karu approved animal studies.

ETHICAL APPROVAL

All procedures were conducted in accordance with the ethical guidelines for the use of animals in research, and the study protocol was approved by the Department of Medical Laboratory Science, Bingham University, Karu; with no: BHU/FOAHS/DML/02/04/2024.

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 Nasarawa state, Nigeria. This herbal mixture has NAFDAC REG NO: A7-0804L and constitutes a mixture of the following herbs: Vernonia amygdalina, Saccharum officinarum, Allium sativum, Cajanus cajan, Caramel, and Zingiber officinale.

EXPERIMENTAL ANIMALS

The Wistar rats were acclimatized for 2 weeks before commencement of the experiment. On the third week, the Wistar rats were divided into three groups 1,2 and 3. Four rats were assigned to each group. Group 1 was the control group and were fed with the normal rat chow and water *ad libitum.* Group 2 and 3 were administered different dosage of the herbal cleanser, 0.5ml/ body weight for medium dosage and 1ml/ body weight for high dosage respectively for 21 days. All rats were weighed prior to the commencement of administration, and subsequently weighed weekly (once a week) using weighing balance.

SAMPLE COLLECTION AND PREPARATION

Following the 21 days of extract administration, the animal’s body weight was recorded using a weighing balance (CS 200, China). The animals were euthanized and blood samples were collected via cardiac puncture into EDTA bottles.

HAEMATOLOGICAL INVESTIGATION

The red blood cell count (RBC), white blood cell (WBC), platelets (PLT), hemoglobin concentration (HGB), hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell

hemoglobin concentration (MCHC) were estimated using an auto-analyzer machine (Genesis HA6000) according to the protocol from the manufacturer.

STATISTICAL ANALYSIS

The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 27. Descriptive statistics such as the mean and standard deviation were calculated. The one-way ANOVA were employed to examine the differences in mean values across different groups. The Pearson's correlation coefficient was used to determine the association between variables. A p-value less than 0.001 was considered statistically significant for all tests.

RESULTS

Table 1 and Figure 1 presents the changes in the mean body weight of rats in different bitter extra dosage groups (Control, 0.5ml, and 1ml) across four time points (Initial, Week 1, Week 2, and Week 3). At the start of the experiment, 1ml Group had the highest average body weight (134.68 ± 7.68), followed by 0.5ml (112.40 ± 8.39), while the Control group had the lowest (93.85 ± 4.51). The differences in body weight between the groups were statistically significant (F(2) = 107.06, p-value < 0.001).

Over time, all groups showed an increase in body weight, with 1ml group continuing to have the highest average weight throughout the study. By Week 3, 1ml Group had an average weight of 178.93 ± 12.59, significantly higher than 0.4ml Group (163.25 ± 11.28) and the Control group (130.35 ± 6.47). The weight differences over time were also statistically significant F (3) = 55.54, p-value = 0.000), demonstrating that the weight gain was consistent across all groups during the study period.

Table 1: Changes in the Weight of Rats over Time for Different Treatment Groups

TIME	Control (Mean ± SD)	0.5ml Group (Mean ± SD)	1ml Group (Mean ± SD)	Total (Mean ± SD)
Initial	93.85 ± 4.51	112.40 ± 8.39	134.68 ± 7.68	113.64 ± 18.57
Week 1	103.03 ± 5.33	133.93 ± 5.40	151.50 ± 11.79	129.48 ± 22.17
Week 2	121.90 ± 4.48	152.28 ± 10.33	171.70 ± 14.13	148.63 ± 23.39
Week 3	130.35 ± 6.47	163.25 ± 11.28	178.93 ± 12.59	157.51 ± 23.16
Total	112.28 ± 15.73	140.46 ± 21.54	159.20 ± 20.80	137.32 ± 27.29
	F-Statistics	D f	p-value	
Group	107.06	2	0.000*	
Time	55.54	3	0.000*	

*Two-way ANOVA; Significant at ≤0.001

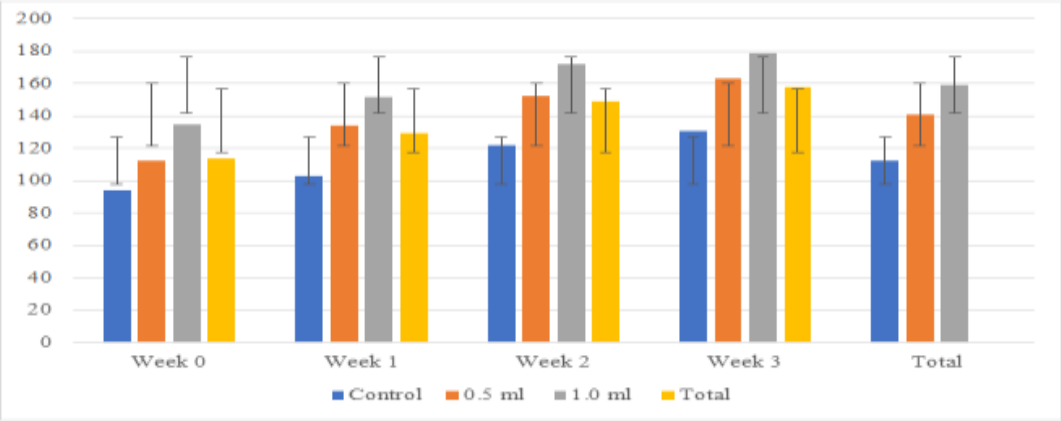

Fig 1: Changes in the Weight of Rats over Time for Different Treatment Groups

Table 2, Figure 2 and 3 shows the comparison of blood parameters among different rat groups treated with bitter extra. The results reveal that RBC levels were significantly higher in 0.5ml Group (8.83 ± 0.26 ×10⁶/µL) compared to 1ml Group (8.10 ± 0.64 ×10⁶/µL) and the control group (6.70 ± 0.81 ×10⁶/µL) (F(2) = 12.33, p = 0.003). Similarly, platelet counts were significantly higher in 0.5ml Group (572.25 ± 93.43 ×10³/µL) compared to 1ml Group (566.25 ± 38.79 ×10³/µL) and the control group (349.50 ± 39.62 ×10³/µL) (F(2) = 16.37, p = 0.001). Mean corpuscular volume (MCV) was significantly lower in 0.5ml Group (55.00 ± 0.00 fL) and 1ml Group (56.75 ± 1.89 fL) compared to the control group (64.50 ± 1.73 fL) (F(2) = 46.60, p = 0.000). Likewise, mean corpuscular hemoglobin (MCH) was significantly lower in 0.5ml Group (18.05 ± 0.17 pg) and 1ml Group (18.73 ± 0.41 pg) compared to the control group (21.13 ± 1.02 pg) (F(2) = 25.11, p <0.001).

Table 2: Comparison of Hematological Parameters among Different Groups Treated with Bitter Extra

Parameter	Control (Mean ± SD)	0.5ml Group (Mean ± SD)	1ml Group (Mean ± SD)	F-value	df	p-value
WBC (×10³/µL)	8.30 ± 3.19	7.20 ± 2.92	4.70 ± 0.73	2.12	2	0.176
RBC (×10⁶/µL)	6.70 ± 0.81	8.83 ± 0.26	8.10 ± 0.64	12.33	2	0.003*
HB (g/dL)	14.13 ± 1.34	15.95 ± 0.59	15.15 ± 0.90	3.41	2	0.079
PCV (%)	43.00 ± 4.24	48.50 ± 1.73	46.00 ± 2.16	3.55	2	0.073
PLT (×10³/µL)	349.50 ± 39.62	572.25 ± 93.43	566.25 ± 38.79	16.37	2	0.001*
N (%)	17.50 ± 7.85	23.50 ± 10.97	20.50 ± 5.45	0.51	2	0.617
L (%)	74.50 ± 5.97	71.50 ± 9.75	72.00 ± 6.68	0.18	2	0.841
M (%)	4.50 ± 1.29	2.50 ± 0.58	4.25 ± 1.26	3.98	2	0.058
E (%)	3.50 ± 3.11	2.00 ± 0.82	2.25 ± 1.26	0.65	2	0.545
B (%)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	-	-	-
MCV (ft)	64.50 ± 1.73	55.00 ± 0.00	56.75 ± 1.89	46.60	2	0.000*
MCH (pg)	21.13 ± 1.02	18.05 ± 0.17	18.73 ± 0.41	25.11	2	0.000*
MCHC (g/dL)	32.85 ± 1.07	32.80 ± 0.22	32.95 ± 0.41	0.05	2	0.950

*One-way ANOVA; Significant at ≤0.001

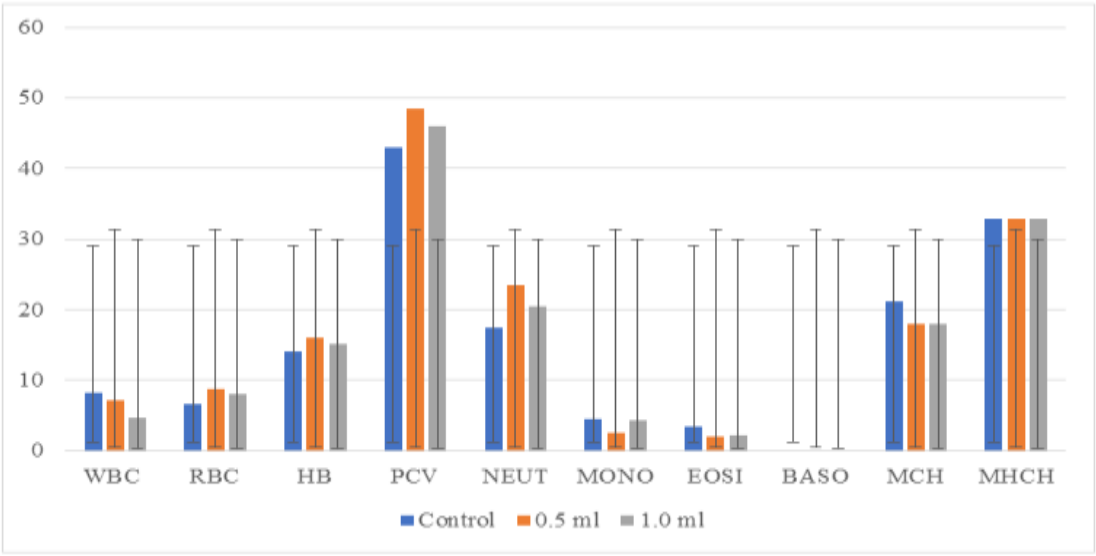

Fig 2: Comparison of Hematological Parameters among Different Groups Treated with Bitter Extra

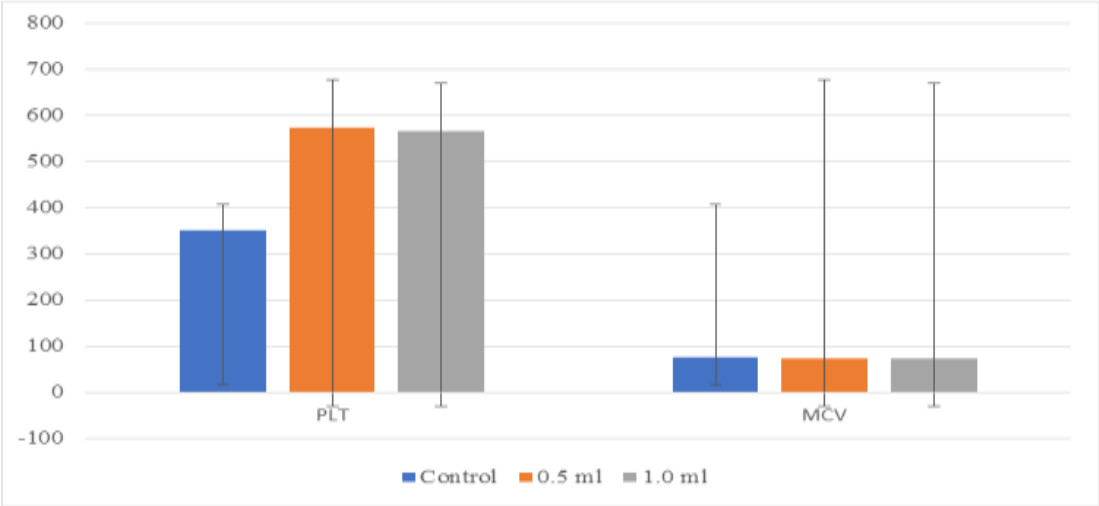

Fig 3: Comparison of Platelets and MCV Parameters among Different Groups Treated with Bitter Extra

Table 3 presents the bivariate correlations between blood parameters. The analysis indicates a statistically significant negative correlation between WBC and both platelet count (r = -0.623, p = 0.030) and neutrophils (r = -0.610, p = 0.035). RBC also demonstrated a strong negative correlation with monocytes (r = -0.777, p = 0.003), a trend similarly observed between hemoglobin and monocytes (r = -0.771, p = 0.003), PCV and monocytes (r = -0.786, p = 0.002), and between neutrophils and lymphocytes (r = -0.968, p = 0.000). Additionally, MCV showed significant negative correlations with RBC (r = -0.944, p = 0.000), hemoglobin (r = -0.777, p = 0.003), PCV (r = -0.783, p = 0.003), and platelet count (r = -0.875, p = 0.000). Similarly, MCH was inversely correlated with RBC (r = -0.917, p = 0.000), hemoglobin (r = -0.715, p = 0.009), PCV (r = -0.774, p = 0.003), and platelet count (r = -0.891, p = 0.000).

On the positive side, RBC was strongly correlated with hemoglobin (r = 0.930, p = 0.000), PCV (r = 0.940, p = 0.000), and platelet count (r = 0.764, p = 0.004). Additionally, eosinophils were positively correlated with WBC (r = 0.642, p = 0.024), and there was a strong positive association between hemoglobin and PCV (r = 0.974, p = 0.000) as well as between PCV and platelet count (r = 0.586, p = 0.045).

Table 3: Correlation between Blood Parameters

Parameters	R-value	P-value
WBC vs RBC	-0.124	0.702
WBC vs HB	0.145	0.653
WBC vs PCV	0.051	0.876
WBC vs PLT	-0.623	0.030*
WBC vs N	-0.610	0.035*
WBC vs L	0.572	0.052
WBC vs M	-0.063	0.847
WBC vs E	0.642	0.024*
RBC vs HB	0.930	0.000*
RBC vs PCV	0.940	0.000*
RBC vs PLT	0.764	0.004*
RBC vs N	0.369	0.237
RBC vs L	-0.316	0.317
RBC vs M	-0.777	0.003*
RBC vs E	-0.032	0.922
HB vs PCV	0.974	0.000*
HB vs PLT	0.535	0.073
HB vs N	0.292	0.356
HB vs L	-0.294	0.354
HB vs M	-0.771	0.003*
HB vs E	0.229	0.474
PCV vs PLT	0.586	0.045*
PCV vs N	0.349	0.266
PCV vs L	-0.316	0.317
PCV vs M	-0.786	0.002*
PCV vs E	0.109	0.736
PLT vs N	0.547	0.065

PLT vs L	-0.464	0.129
PLT vs M	-0.555	0.061
PLT vs E	-0.373	0.232
N vs L	-0.968	0.000*
N vs M	-0.397	0.201
N vs E	-0.450	0.142
L vs M	0.361	0.249
L vs E	0.276	0.385
M vs E	-0.217	0.498
MCV vs WBC	0.317	0.315
MCV vs RBC	-0.944	0.000*
MCV vs HB	-0.777	0.003*
MCV vs PCV	-0.783	0.003*
MCV vs PLT	-0.875	0.000*
MCH vs WBC	0.387	0.214
MCH vs RBC	-0.917	0.000*
MCH vs HB	-0.715	0.009*
MCH vs PCV	-0.774	0.003*
MCH vs PLT	-0.891	0.000*
MCHC vs WBC	0.383	0.219
MCHC vs RBC	-0.022	0.946
MCHC vs HB	0.134	0.678
MCHC vs PCV	-0.091	0.778
MCHC vs PLT	-0.200	0.533

*Pearson Correlation; Significant at ≤0.05

DISCUSSION

The results of this research shed light on the impact of varying dosages of bitter extract on body weight and blood parameters in rats, emphasizing how physiological responses fluctuate based on the dosage. The study observed a distinct pattern of weight gain in all experimental groups, with the group receiving the 1 ml bitter extract dosage exhibiting the most pronounced increase in body weight throughout the experiment. Conversely, the control group consistently showed the least amount of weight gain, indicating that the administration of the bitter extract was likely responsible for the observed weight gain in the treated rats. These findings align with earlier research, although some studies have reported differing outcomes. For instance, previous investigations have found certain herbal extracts to be associated with increased metabolic activities and enhanced nutrient absorption, both of which could contribute to weight gain [12]. On the other hand, some authors suggests that bitter extract compounds might have an appetite-suppressing effect, depending on the specific type of extract used and the dosage administered [13, 14]. In a similar vein, a systematic review of the effects of Citrus aurantium (commonly known as bitter orange) extract on body weight presented varying results. Some studies within the review reported weight loss as an outcome, while others either found no significant change or even recorded weight gain in subjects [15].

The notable weight gain observed in the group receiving a 1ml dosage of the bitter extract, when compared to both the 0.5ml dosage and control groups, highlights a clear dose-dependent effect of bitter extra on body weight. This dose-dependent response suggests that as the amount of the bitter extra administered increases, there is a corresponding increase in its impact on weight gain. The mechanisms behind this could involve the extract enhancing the efficiency of metabolic pathways, potentially increasing fat storage, or stimulating appetite, particularly at higher dosages. Therefore, for individuals taking bitter extra for its purported health benefits, particularly in areas such as weight management or metabolic regulation, it becomes crucial to pay careful attention to dosage. The potential risks of unintended weight gain, especially at higher dosages, underscore the importance of precise dosing to ensure beneficial outcomes without adverse effects. Consequently, appropriate dosing protocols would be essential for optimizing the therapeutic use of bitter extract in various health contexts.

Existing research has demonstrated that consuming toxic herbal products can lead to changes in the hematological profile, and drugs that have toxic effects can result in organ damage as well as significant alterations in hematological biomarkers [16]. Assessing hematological parameters is essential as it provides key physiological insights into the overall condition of the blood and can help in diagnosing various health issues. In the

present study, the group receiving a moderate dosage of the bitter extra displayed a higher red blood cell (RBC) count compared to both the group receiving a higher dosage and the control group. This observation suggests that moderate doses of the extract may stimulate erythropoiesis, which is the process of red blood cell production. This effect could be attributed to the presence of bioactive compounds in the extra that promote blood cell generation, suggesting potential therapeutic applications in treating anemia or other blood-related disorders.

A similar trend was observed with platelet counts, where the moderate dosage exhibited significantly elevated platelet levels compared to the control and higher dose groups. This indicates that the bitter extract might also play a role in enhancing platelet production, which could be beneficial in managing conditions involving low platelet counts. However, in the group receiving the higher dosage, platelet levels were slightly reduced compared to the moderate dosage group, implying that the beneficial effects of the extract might plateau or diminish at higher doses. This pattern is consistent with findings from other studies that have shown that herbal supplements tend to have optimal effects at moderate dosages, whereas higher dosages can lead to reduced efficacy or even adverse effects [17, 18].

Interestingly, both the moderate and higher dosage groups showed lower mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) values compared to the control group, indicating changes in the characteristics of the red blood cells. Lower MCV and MCH values are typically associated with smaller, more densely packed red cells, which could represent an adaptive response to the intake of the extract. However, the precise implications of these changes, particularly regarding the overall functionality and health of the red blood cells, remain unclear and require further investigation. A significant reduction in MCV can be indicative of several conditions, such as the production of unhealthy RBCs with abnormal size and shape, an increase in WBCs, or deficiencies in essential vitamins and minerals like iron, or even overhydration [19, 20]. This suggests that at higher doses, the bitter extract may have negatively impacted the production of healthy red blood cells with normal size and shape. Specifically, the RBCs produced in the higher dosage group appeared to have a greater proportion of smaller erythrocytes, which are densely packed, suggesting the presence of iron deficiency, potentially leading to hypochromic microcytic anemia [21].

Despite these changes in RBC parameters, the study found no statistically significant differences in WBC counts across the various dosage groups. This finding aligns with the results of other studies, which also did not report any significant impact of bitter extracts on most blood parameters, including WBC counts, under different dosages [22, 23]. This suggests that while bitter extracts may affect certain hematological parameters like RBCs and platelets, their impact on WBCs may be minimal or negligible across a range of dosages.

The correlation analysis identified several important relationships between blood parameters. One key observation was the inverse association between white blood cell (WBC) counts and both platelet levels and neutrophils, implying that as immune markers decline, there may be a compensatory rise in platelet production. Additionally, a strong inverse correlation was found between red blood cell (RBC) counts and monocyte levels, suggesting that an increase in RBC production could lead to a reduction in inflammatory activity. This points to a potential anti-inflammatory effect of the bitter extract at moderate doses, aligning with previous research on herbal remedies with known anti-inflammatory effects [17]. Likewise, strong negative correlations were noted between mean corpuscular volume (MCV) and RBC, hemoglobin, and platelet counts, suggesting that as red cell size decreases, their

concentration and hemoglobin levels are more finely tuned. This may indicate an adjustment in red blood cell efficiency in response to the bitter extract, reinforcing the idea of dose-dependent hematological effects caused by the extract.

The limitation of the study is centered on hematology parameters due to cost, however, researchers could explore the impacts of Goko herbal cleanser mixture on the histology of the tissues and biochemistry of Wistar rat.

CONCLUSION

Goko herbal cleanser mixture elicit a dose-dependent effect on erythropoiesis and thrombopoiesis, highlighting the need for careful dosage regulation to maximize therapeutic usefulness and outcome, and determining its risks is the major contribution of the study; enhancing a better understanding of herbal supplements. Hence, further research is needed to determine its safety and efficacy in humans.

AUTHORS CONTRIBUTIONS:

A S P The principal investigator: responsible for research concept and selection of research title, wrote the research protocol and proposal; analyzed and collated the research data; also carried out the statistical analysis of the data along with the second author, documented and interpreted the data; wrote the manuscript.

ASM and GD involved in collation and analysis of data and proofreading.

CJJ and O H B, involved in the selection of the research title and supervision of the research work and carried out statistical analysis of data. All the authors read and approved the manuscript for publication. All authors read and agreed to the publication of the manuscript.

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Conflict of Interest: None

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