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EVALUATION OF HAEMATOLOGICAL AND BIOCHEMICAL CHANGES IN *PLASMODIUM BERGHEI*-INFECTED MICE TREATED WITH LEAF EXTRACT OF *Cassia sieberiana* AND *Chromolaena odorata*

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Abstract
The haematological and Biochemical effect of *Cassia sieberiana* and *Chromolaena odorata* leaf extract on mice infected with *Plasmodium berghei* was evaluated. 30 Swiss albino mice (23-32g) were divided into 6 groups of five per group. Groups PC, CC_1, CC_2, CC_3, and CC_4 were infected with blood containing the parasite. Group NC was not infected and served as the normal control. On the 5th day after infection, the mice in each group were treated. Mice in Groups CC_2, CC_3 and CC_4 were administered orally with 250, 500 and 1000 mg/kg body weight of *Cassia sieberiana* and *Chromolaena odorata* leaf respectively for five days. Group PC was not treated while Group NC was given distilled water. Group CC_1 was treated with 10 mg/kg body weight of chloroquine. After treatment, these mice were sacrificed and blood samples were collected for haematological and biochemical analysis. The result of the combined leaf extract on the haematological parameter indicated that packed cell volume, haemoglobin and red blood cell, were significantly increased by the extract in a dose-dependent mode, while White blood cell, monocytes, neutrophils and lymphocytes were significantly ($p<0.05$) reduced by the extract in a dose-dependent manner when compared to the parasitized untreated group. The level of aspertate transferase, alanine transferase, total protein concentration, urea and creatinine, alkaline phosphate and total bilirubin in all the mice infected with the parasite significantly ($p < 0.05$) increased. However, on the administration of the extract it was reduced in the treated groups. The reduction in the levels of these enzymes is an indication that *Cassia sieberiana* and *Chromolaena odorata* have no hepatotoxic effect on the mice at the dose levels administered.

Keywords: Haematology, Biochemical, Cassia sieberiana, Chromolaena odorata,Plasmodium berghei

Introduction

Plants have been employed as references of healthcare for a variety of diseases for an extremely long period, Due to the species diversity and probably because of their abundant supply of natural and phytochemical compounds (Farombi, 2003). In rural areas where access to modern healthcare facilities is constrained by the level of development plants and herbs continue to be the mainstay (WHO, 2014). Greater certainty about their use has resulted from the extraction of several drugs and chemotherapeutics from medicinal plants as well as from commonly used herbal remedies in industrialized societies (Sofowora et al., 2013). In Sub-Saharan Africa where malaria is still a major problem, it has become a widespread scourge and a global public health issue, claiming millions of lives annually and degrading the quality of life for many others (WHO, 2011). In the tropics, where it is estimated to cause up to 1.2 million fatalities annually and 200 million clinical cases, it is still the most serious parasitic disease (Murray et al., 2012). Each year, it affects hundreds of millions of people, mostly in Sub-Saharan Africa, Asia, and South America where young children and expectant mothers are particularly vulnerable (WHO, 2019).

A reliable tool such as biochemical indices has been used to evaluate animal health status (Saxena et al., 2011). Malaria-related changes in biochemical indices alterations are linked to cellular changes in metabolic activities,

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heme metabolism, membrane lipid peroxidation, and stress enzymes (Asagba et al., 2010). Several haematological indices, such as haemoglobin, white and red blood cells, mean cellular volume, platelet count, and haemoglobin factor, have been linked to changes in malaria incidence (Adedapo et al., 2007; Nsiah et al., 2010; Bakhubaira, 2013). Alteration in biological functions of the liver and metabolic disturbance related to electrolyte and fluid instability is a common disorder of malaria, and this disorder changes depending on the parasitemia level (Nsonwu-Anyanwu et al., 2017). Malaria infection have been shown to alter plasma biochemical indicators (Momoh et al., 2014). Studies show that blood with a malaria parasite in it undergoes biochemical and haematological changes, and typical complications of this disease are also present (Warimwe et al., 2013). One of the hematopoietic alterations connected to protozoal infection is circularised intravenous clotting, along with inflammatory diseases, anaemia, and others (Al-Salahy et al., 2016). Changes in the physical and chemical characteristics of plasma that has been contaminated with the malaria parasite can depend on several factors, including the degree of malaria endemicity, nutritional requirement, the appearance of hemoglobinopathies, sociodemographic characteristics, and level of malaria immunity (Al-Salahy et al., 2016). Hence, individual wellness can be evaluated by exploiting a variety of haematological parametric quantities. The monetary value of confronting mosquito bites by using a rebarbative, chemical substance, or aerated mosquito nets is expensive for a group of an individual surviving in the malaria-endemic domain throughout sub-Saharan Africa (WHO, 2015). One difficulty obstructing the management of malaria is the resistance to the best-selling, low-priced, and assured first-line malaria treatments, such as Fancider and Chloroquine (Winstanley et al., 2005). Schemes for managing malaria vectors are difficult because they show resistance to several insecticides (WHO, 2017). The third and most imperative problem is the widespread industry production of faux anti-malarial medicine. The fourth and least pressing trouble is the deficiency of the indispensable substructure and resources to negotiate and control malaria distribution and armed combat bogus remedy. (Sendagire et al., 2005). According to medical studies on a significant figure of patients, artemisinin is efficacious in emptying disease activity and lowering evidence in sick people with malaria, including several with chloroquine-resistant malaria and cerebral malaria (Woodrow et al., 2005). Notwithstanding, artemisinin seems to have an adverse reaction as well as being unproductive when it is used on its own. Non-synthetic prescription drugs are required to be investigated for and to substitute artemisinin treatment. Researchers must assess or perform additional scientific studies on the biochemical and haematological criteria to establish the effectiveness and overall security of *Cassia sieberiana* and *Chromolaena odorata* in *Plasmodium berghei*-infected mice.

Materials and Methods

The plants were obtained from the Rumuolumeni wild area with a latitude of 4.49'4" and a longitude of 6.51'24". Which is located in Obio/Akpor Local Government Area of Rivers State, Nigeria. The plants were deposited in a botanical garden where a plant taxonomist identified them. The technique for the ethanolic infusion was modified slightly from that of Blight and Dyer (1958). In a glazed ceramic jar, freshly cleaned *C. sieberiana* and *C.odorata* leaves were grinded. The combined leaf paste was extracted with absolute ethanol and left to sit overnight before being filtered through cellulose paper. To determine the dry weight of the leaves residue, the filtrate was congregated in a turning evaporator at (45°C) and the substance was dehydrated beneath ablated pressure. The weight of the substance was ascertained and the output proportion was calculated. The infusion was in good order in an air-binding container in a refrigerator until required. The experiment received a favourable reception from the ethics board of Ignatius Ajuru University of Education, Port Harcourt. The animal house of the Faculty of Basic Medical Science, University of Port Harcourt, Rivers State, Nigeria, sold thirty adult Swiss mice that were healthy and weighed (23–33g), along with mice that were already infected with *P. berghei*. The guidelines for the care and use of laboratory animals published by the National Research Council of the United States of America were followed when handling the mice during the two-week period in which they were housed and trained to adapt to the laboratory environment. They had access to water and were fed by essential feed growers (NIH, 2011).

On the third day after inoculation of the parasite clinical signs of malaria infection were noticed in the mice. The *P. berghei* parasitized mice were anaesthetized in a glass jar with cotton wool that had been drenched in trichloromethane. Blood was collected from the anaesthetized mice by puncturing their hearts with sterile strings and needles. Each of the mice was infected for the experiment using 0.2 ml of infected red blood cells administered

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intraperitoneally. The blood was diluted in saline solution in a magnitude relation of 1:10, or 1 ml of blood in 10 ml of solution.

Six groups of five adult Swiss mice each were formed from the 30 overall mice. NC, PC, CC_1, CC_2, CC_3, and CC_4 were the designations acknowledged to the groups. The NC normal control group did not receive any parasite inoculation; it was only fed food and distilled water. Unlike the CC_1 control group, which received a parasite inoculation and 10 mg/kg of chloroquine as a treatment, PC did not receive any medication in addition to the parasite inoculation. The dosages of 250, 500, and 1000 mg/kg body weight for the combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata* were given over 4 days to Groups CC_2, CC_3, and CC_4. After five days of receiving medication, mice from each group became unconscious, and blood samples from the heart of the mice were taken and retained in a tagged specimen container for assessment. To get an accurate result, the haematological parameters for this experimentation were constituted by exploiting the accepted method. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were ascertained by adopting the method of Reitman and Frankel (1957) while the serum alkaline phosphatase (ALP) activity was deduced by the enzymatic colourimetric method by Wright et al. (1972). The biuret method was followed in determining the protein concentration (Gonall et al., 1949). Data are presented as Mean + Standard Error of Mean (+SEM) for data expression. With the SPSS (Statistical Package for Social Sciences) Version 20.0, ANOVA was used to compute the statistical significance. A p-value of 0.05 or less indicates statistical significance.

Results

Effect of the combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata* on hematological parameters of mice.

Reduced packed cell volume (PCV) was seen in the untreated group of mice according to their haematological test. Moreover, when compared to the untreated group, the extract at 1000mg/kg significantly $p< 0.05$ increased.When in comparison with the untreated control group, the mice haemoglobin (HB) levels significantly increased $p< 0.05$ in a dose-dependent manner after extract administration. Red blood cells (RBC) revealed a reduction in the untreated group when compared to the normal control. Along with a gradual increase in dose concentration, the treated groups also experienced a restoration of order in the RBC. In the case of the group that received no treatment, the total White blood cell (WBC) count was significantly higher. The extract decreased WBCs at 1000mg/kg, which was comparable to CQ at $p<0.05$. When compared to the parasitized, untreated group, the combined extract at 250, 500, and 1000mg/kg greatly reduced neutrophils (NEU), lymphocytes (LYM), and monocytes (MON) in the treatment group at a significant level of $p<0.05$ (Table 1).

Table 1: Effect of the combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata* hematological parameters of mice.

Treatment	PCV	HB	RBC	WBC	NEU	LYM	MON
NC	49.75±1.79	15.02±0.26	4.85±0.12	3.35±0.19	73.25±1.31	23.00±0.40	1.00±0.40
PC	19.00±1.08^a	5.82±0.49^a	2.12±0.14^a	12.10±0.72^a	98.57±2.56^a	31.75±1.70^a	2.50±0.28^a
CC_1	43.**50**±1.55^b	13.80±0.23^b	4.42±0.14^b	5.20±0.09^b	72.75±1.03^b	21.25±0.48^b	0.50±0.28^b
CC_2	37.00±1.15^c	12.63±0.17^c	4.33±0.03^c	5.03±0.08^c	74.33±2.33^c	18.00±1.00^c	0.33±0.33^c
CC_3	44.67±1.45^b	14.87±0.21^d	4.63±0.21^d	4.53±0.17^d	75.67±1.67^d	20.67±0.33^d	0.66±0.67^d
CC_4	49.33±0.88^d	15.13±0.24^e	5.23±0.37^e	4.20±0.11^e	76.67±1.45^e	20.66±0.67^d	0.93±0.33^e

Data as mean ± SEM (Standard error of the mean). Values with different superscripts down the column significantly differ at $p<0.05$. NC: Normal control, PC: Parasitized Control; CC_1: Positive control (10mg/kg of Chloroquine), CC_2: 250mg/kg of combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata*, CC_2: 500mg/kg of combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata,* CC_3: 1000mg/kg of combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata.*

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Effect of the combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata* on biochemical parameters of mice.

Following treatment with the leaf extract, the mice biochemical indices showed a significant decline in Aspertate transferase (AST), Alanine transferase (ALT), Alkaline phosphate (ALP), Total protein concentration (TPC), Total bilirubin concentration (TBC), and Urea concentration (URE) compared to the untreated group of mice. When compared to the parasitized untreated group, lower levels of the parameters were found in the treated groups with 250, 500, and 1000mg/kg. Notwithstanding, a marked decline was observed in 1000mg/kg, which was at par with CQ at $P< 0.05$. (Table 2).

Table 2: Effect of the combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata* on the biochemical parameter of mice

Treat-ment	AST(u/l)	ALT(u/l)	ALP(u/l)	TP(g/dL)	TB(g/dl)	URE(mg/dl)	CRE(mg/dl)	Uric acid mg/dL
NC	10.00±0.57	11.00±1.15	118.67±1.45	69.33±1.45	5.67±0.33	2.43±0.12	60.67±2.33	3.09±0.14
PC	11.67±10.34[a]	12.00±5.57[a]	119.33±2.30[a]	70.67±5.23[a]	6.00±5.13[a]	4.23±0.93[a]	62.33±3.17[a]	4.4±00.10[a]
CC_1	10.33±8.68[b]	11.33±5.10[b]	118.00±2.64[b]	69.33±4.48[b]	5.67±2.96[b]	2.10±0.56[b]	60.33±3.84[b]	3.15±0.17[b]
CC_2	11.00±1.52[b]	11.33±2.40[b]	118.33±2.33[b]	69.00±0.58[b]	5.00±0.57[b]	2.10±0.05[b]	60.33±1.20[b]	3.38±0.20[b]
CC_3	10.00±1.15[b]	10.33±1.45[b]	118.67±1.85[b]	69.00±1.53[b]	5.00±0.58[b]	2.83±0.09[b]	60.67±0.89[b]	3.16±0.17[b]
CC_4	10.00±1.15[b]	18.67±2.40[b]	118.33±2.91[a]	69.33±0.67[b]	5.33±1.20[b]	2.17±0.09[b]	60.00±1.15[b]	3.10±0.15[b]

Data as mean ± SEM (Standard error of the mean). Values with different superscripts down the column significantly differ at $p<0.05$. NC: Normal control, PC: Parasitized Control; CC_1: Positive control (10mg/kg of Chloroquine), CC_2: 250mg/kg of combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata*, CC_2: 500mg/kg of combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata*, CC_3: 1000mg/kg of combined leaf extract of *Cassia sieberiana* and *Chromolaena odorata.*

Discussion

In this study, the leaf extract of *Cassia sieberiana* and *Chromolaena odorata* significantly normalized the distorted haematological indices in PCV, HB, WBC, RBC, NEU, LYM, and MON when compared to the negative control at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. To identify any possible hemolysis and anaemia introduced by *falciparum* malaria, PCV is scrutinized. Nevertheless, the PCV was controlled to a level that was nearly normal in the treated groups. PCV significantly decreased in the untreated group, as expected. The dominant signs of recovery were seen in the group receiving 1000 mg/kg/day of the infusion. It has been widely established that *P. falciparum* tends to produce free radicals, which have the possibility of destroying red blood cell membranes. PCV may have decreased for a variety of reasons, this being just one of them.

Therefore, the primary cause of the extract ability to maintain PCV in infected mice may be its antioxidant activities in phytoconstituents. In addition, the extract increased iron content may have significantly aided in red blood cell formation. This is in line with the study of (Gitua et al., 2012). A significantly low haemoglobin level is a reliable sign of anaemia (Mengiste et al., 2012). The study revealed that, when compared to the negative control group, the haemoglobin values of the extract-treated groups significantly increased after treatment. Carroll (2014) asserts that erythropoietic inhibition, clearance of non-infected RBC, and obliteration of diseased RBC all have been associated with both mouse and human *P. falciparum* nutritional deficiencies. According to the study, there was a noticeable increase between the treated group of mice and the untreated control group. Severe infection and anaemia, which the extract effectiveness was capable of relieving, have been underlined by the declining trend in the untreated group. The study supports the conclusions made by Elele et al. (2021) who reported a significant decrease in the RBC of the untreated mice group but the treated groups were able to restore the RBC to normal. The treated groups WBC decreased substantially when measured against the control group. WBCs elicit antibodies

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which fight off infection regularly, and quickly and safeguard the immune system from invasion by invading pathogens through phagocytosis, and combat infectious diseases (Mojiosola et al., 2013). This shows that the mice given the extract at doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg appeared to be more productive in warding off the malaria parasite. Despite the WBC increase in the extract-treated group at a dose of 1000 mg/kg, this could suggest that a dose of 1000 mg/kg of the extract is safe, which would support the report of (Nworgu et al., 2022). In comparison to the treated group, the study assessment of the neutrophils demonstrated a decrease in the infected, untreated group, indicating the presence of malaria infection, which is consistent with the report (Ekvall, 2003).

The untreated group had high levels of lymphocytes and monocytes, which were visible. Despite a WBC increase in the extract-treated group at a dose of 1000 mg/kg, this could suggest that a dose of 1000 mg/kg of the extract is safe, which would support the report (Nworgu et al., 2022). In comparison to the treated group, the study assessment of the neutrophils demonstrated a decrease in the infected, untreated group, indicating the presence of malaria infection, which is consistent with the earlier report (Ekvall, 2003). The untreated group had high levels of lymphocytes and monocytes, which were visible. Due to the limited time, the plant extract was strong enough to restore them to the reference range. It could be said that the phytochemicals in the plant extract, such as phenol, saponins, flavonoids, and glycoside, which have antioxidant effects, are also what exacerbated the lower proportion of monocytes in the extract-treated groups (Omonkhua et al., 2013). The increase in lymphocytes found in this study could have a detrimental effect on the immune system effector cells. The fact that the extract at 1000mg/kg dose effectively reduces neutrophils could indicate that the extract can help blood components endure phagocytosis.

Alkaline phosphate, aspertate transferase, and alanine transferase enzymes were identified in this study and their activities were normalized by the administration of *C. sieberiana* and *C. odorata* leaf extract. These enzymes may demonstrate a health condition if their blood levels are elevated (Malakout et al., 2017). The AST and ALT test is most often used to diagnose liver problems, track severe deficiency, evaluate the beneficial effects of treatment, and make sure that drugs are not negatively affecting the liver. Upon receiving a *P. berghei* injection, the AST of the infected mice significantly increased. Due to *P. berghei* infection, the hepatocytes have recently completed degenerative changes which have additionally altered the enzyme activities. Following treatment, it was discovered that the levels of AST and ALT in the group that was not treated with any extract were significantly lower $p< 0.05$ than those in the group that had received extract treatment. Momoh et al. (2014) and Nworgu et al. (2022) speculate that the extracts may have activated the enzyme, which might account for the decrease in the treated groups.

The untreated control group showed higher levels of ALP when compared to the groups treated with various extract concentrations. The liver damage that is associated with this rise in ALP was mildly resolved in the group receiving both the extracts and the standard medication. The outcome is consistent with Momoh and Manuwa (2014) and Gboeloh (2016). The total protein level of the Control group dropped after treatment, compared to the other study groups. A decrease in protein synthesis could be the root of this. These results confirmed earlier research that suggested chronic conditions, like malaria, are life-threatening infections that potentially lower the production of proteins by breaking down the cells considered necessary for it (Yavuzsen et al., 2005). In the present study, compared to the groups that received different concentrations of the extracts, plasma creatinine massively increased ($P<0.05$) due to the damage caused by the parasite's ongoing multiplication. The total bilirubin level in the untreated group was also discovered to be higher than it was in the healthy control group due to the infection. The level of TB progressively dropped as the dose of extract was increased. This is in line with the research done by Nworgu et al. (2022). The investigation has revealed that the urea levels were considerably lower in the infected untreated groups than those in the dose-treated groups after receiving doses of 250 mg/kg, 500 mg/kg, and 1000 mg/kg. The involvement of impaired renal function might well be demonstrated by a significant increase in serum creatinine. In the assessment of total uric acid, each of the groups under investigation demonstrated the same trend.

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Conclusion

The effects of concentration and length of time on the potentials of the *Cassia sieberiana* and *Chromolaena odorata* leaf extract for the treatment of malaria were impressive and demonstrated normalization therapeutic effect to both haematological and biochemical benchmarks. This study discovered that, if the active components are fully exploited, the plant could be characterized into a group of plants with reducing anti-malarial constituents.

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