Original Research Article

Investigation of Biochemical Parameters of *Plasmodium berghei* Infected Mice After Administration of Ethanolic Leaf extract of *Eucalyptus citriodora*

ABSTRACT

The leaf, stem and bark of *Eucalyptus citriodora* are combined for use in the treatment of malaria in Anyigba, North Central, Nigeria. However, no scientific investigations have been carried out to know the effect of this plant on biochemical parameters of experimental mice. Thus, this study evaluated the biochemical parameters of mice infected with *Plasmodium berghei* after the administration of ethanolic leaf extract of *E citriodora*. Twenty-four (24) mice of body weights between 18-25 g were grouped into six groups. Group 1, infected not treated (negative control), group 2, infected and administered with 0.2 mg/kg of chloroquine (positive control), group 3, not infected, but administered with 0.2 ml of normal saline (normal control), while the remaining three (3) groups were infected and treated with 200, 400 and 800 mg/kg body weight of the extract respectfully. The pack cell volume (PCV) was assessed before and after infection using standard procedure. The mice were sacrificed on the sixth day and blood samples were collected for biochemical analysis using standard methods. The PCV in mice of all groups decrease significantly ($p<0.05$), except group 3 (normal control) that increased. The alanine transaminase (ALT), aspartate transaminase (AST), total bilirubin level was higher in negative control (group 1) than in all other groups studied, but it was higher in the group treated with 200 mg/kg bd wt of the extract than in the positive control and the groups treated with 400 and 800 mg/kg body weight of the extract. A similar trend was observed in the levels of cholesterol, triglycerides, high density lipolipid (HDL) and low density lipolipid (LDL). The level of creatinine, blood urea and nitrogen were observed to be low in groups 1 and 2, compared to other groups. This study revealed that *E. citriodora* ethanolic leaf extract does not exact toxic effect on the internal organs like liver, kidney and heart.

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Keywords: Ethanolic leaf extract, *Eucalyptus citriodora,* Biochemical parameters, *Plasmodium berghei* infected Mice.

INTRODUCTION

Malaria is an endemic communicable disease that is most dangerous in the world and contributed to the major socioeconomic problems which lead to global instability and poverty. It is a disease that manifests as fever, headache, chills, sweating, muscle pain and vomiting (1). Malaria is caused by parasites that are transmitted to humans through the bite of infected female *Anopheles* mosquitoes. Five *Plasmodium* species (*P. falciparum, P. malariae, P. ovale, P. vivax and P. knowlesi*) cause malaria in humans, with *P. falciparum* and *P. vivax* posing the greatest threat (2). Malaria is not transmitted by mosquito alone, but due to the residence of the parasite in red blood cells, it can also be transmitted via blood transfusion, organ transplant, or shared use of needles or syringes contaminated with blood (3). *P. falciparum* is the most prevalent malaria parasite in Africa and accounted for 99 percent cases of malaria in Sub-Saharan Africa in 2016, and was responsible for most malarial deaths globally (4). *P. vivax* is the most common parasite outside of Sub-Saharan Africa, and in 2016, caused 64 percent of cases in the Americas and more than 30 percent in South-East Asia (3).

They are more than 400 different species of *Anopheles* mosquitoes and about 30 are malarial vectors of major importance (3). The two most efficient malarial vectors in the world are *A. gambiae* and *A. funestus* and are the primary malarial vectors in Africa (5). Transmission is more intense in places where mosquito have the long lifespan and where it prefers to bite humans rather than other animals. The long lifespan and strong human-biting habit of the African vector species is the main reason why nearly 90% of the world's malaria cases are in Africa (4).

Over the last 17 years, important measures have been put in place to prevent malaria, leading to 60% reduction in its worldwide death tolls. However, antimalarial drug resistance is a major health problem, which hinders the prevention and treatment of malaria (6). The possible sources of malaria medicines will appear in the use of traditional herbal medicines. Traditional medicines have been the most available, affordable and cheap sources of malaria treatment for most communities (7).

Eucalyptus citriodora Hook (family: Myrtaceae) is a tall, evergreen tree which is cultivated for essential oil, fuel, timbers and medicinal purposes, with the leaves producing fragrant volatile oil with known antibacterial, anti-inflammatory, antiseptic, analgesic, deodorant, diuretic, expectorant activities (8). Also, the leaf contains numbers of phytochemicals including phenolic compounds, flavonoids, aldehydes, ketones and tannins (8). The present study was conducted to evaluate the effect of the ethanolic leaf extract of *E citriodora* on the biochemical parameters of mice infected with *Plasmodium berghe* NK 65.

MATERIALS AND METHODS

Plant Leaf Collection and Extraction

Eucalyptus citriodora leaves were collected in the month of November, 2017 from the premises of the Kogi State University, Anyigba, Nigeria. It was identified and authenticated by an expert and the voucher specimen number of the plant Bio/ FUTA/ 70 was left in the herbarium of the Federal University of Technology, Akure, Ondo State, Nigeria. The leaves were washed, air dried at room temperature for three weeks and pulverized using mortar and pestle. Five hundred grams (500 g) of the pulverized leaf powder was dissolved in 4.5L of 75 % ethanol and allowed to stand in the dark with constant agitation for 72 hours and then filtered using Millipore (pore size 0.7µm) filter paper. The filtrate was concentrated to recover the crude extract using a rotary evaporator at a reduced temperature of 40^{o}C (9).

Phytochemical analysis

Phytochemical analysis of the ethanolic leaf extract of *E. citriodora* was carried out using standard procedures described by Dickson *et al.* (10) and Dada and Oloruntola (9).

Preparation of Leaf Extracts Dosage

The dosages of the extract administered to the mice were prepared by dissolving 0.4, 0.8 and 1.6g of the extract in 20 ml of distilled water each in sterile universal bottle based on the body weight and total number of mice per group to obtain 200, 400 and 800 mg/kg body weight respectively (11).

Source of Experimental Mice and *P. berghei* NK 65

Twenty-four (24) Swiss albino mice of body weight between 18-25 g were obtained from the Animal House, Institute for Advance Medical Research and Training (IMRAT), University College Hospital, University of Ibadan, Nigeria. The animals were housed in cages with saw dust bedding at room temperature and fed with standard diet (Grand cereal) and water ad libitum. They were allowed to acclimatize for 7 days prior to the study. *P. berghei* NK 65 in a donor mouse was obtained from IMRAT.

Infection of Mice with *P. berghei* and Treatments

Twenty-four mice were randomized into six groups of four mice per group. They were infected intravenously with 0.2 ml of $1_{\chi}10^{7}$ standard inoculum of chloroquine sensitive *P. berghei*. Three hours after post infection, 0.2 ml of 200, 400 and 800 mg/kg body weight of leaf extract was administered orally to group 4, 5 and 6 respectively as the treatment dose once daily for four consecutive days. Group 2 (positive control) were treated with 0.2 ml of 5 mg/kg body weight of chloroquine, group 1 (negative control) were given 0.2 ml of normal saline and group 3 (normal control) received 0.2 ml of normal saline but were not infected with *P. berghei.* The treatment was administered for four consecutive days (9).

Determination of Packed Cell Volume

The packed cell volume (PCV) of each mouse was measured before and after infection. Blood was collected, by cutting the tip of the tail of each mouse into heparinized capillary tubes up to ¾ of the entire length. The tubes were sealed by using crystal sealant and placed in a microhaematocrit centrifuge with the sealed ends outwards. The blood sample was centrifuged at 12,000 rpm for 5 minutes. At the end of the centrifugation, the result was read using microhaematocrit reader. The volume of the total blood and the volume of erythrocytes were measured and PCV was calculated as;

PCV = Volume of erythrocytes in a given volume of blood X 100Total blood volume.

Biochemical Assays

Blood was collected from all mice in a lithium heparin bottle through cardiac puncture. The alanine transaminase (ALT), aspartate transaminase (AST), Total bilirubin, Creatinine, Blood Urea Nitrogen, Cholesterol, Triglycerides, HDL and LDL were determined using Spectrophotometer (SM23A).

Statistical Analysis

All data were expressed as mean ±S.E. One-way analysis of variance was used to analyze data. $P < 0.05$ was considered significant difference between means (Duncan's multiple range test).

RESULTS

Phytochemicals present in Ethanolic Leaf Extract of *E. citriodora*

Phytochemical Screening of ethanolic leaf extract of *E. citriodora* revealed the presence of alkaloids, saponins, tannins, anthraquinone, flavonoids and cardiac glycosides (Table 1).

Table 1: Phytochemicals Screening of Ethanolic Leaf Extract of *E. citriodora*

Phytochemicals	Result
Alkaloids	+
Saponins	+
Tannins	+
Anthraquinones	+
Flavonoids	+
Cardiae glycoside	+

Present = + and absent = -

Pack Cell Volume (PCV) Before and After Infection and Treatment

The PCV of groups 1 and 4 (infected untreated and infected treated with 200 mg/kg mice) decreased significantly ($p<0.05$) after 4 days of treatment, similar trends were observed for PCV in mice of groups 5 and 6 (mice infected treated with 400 mg/kg and 800 mg/kg), as well as group 2 (treated with 5 mg/kg chloroquine), but not like those of groups 1 and 4, the decrease was not dosed dependent, but was time dependent. Mice in group 3 (normal control) recorded increases in value of PCV (figure 1).

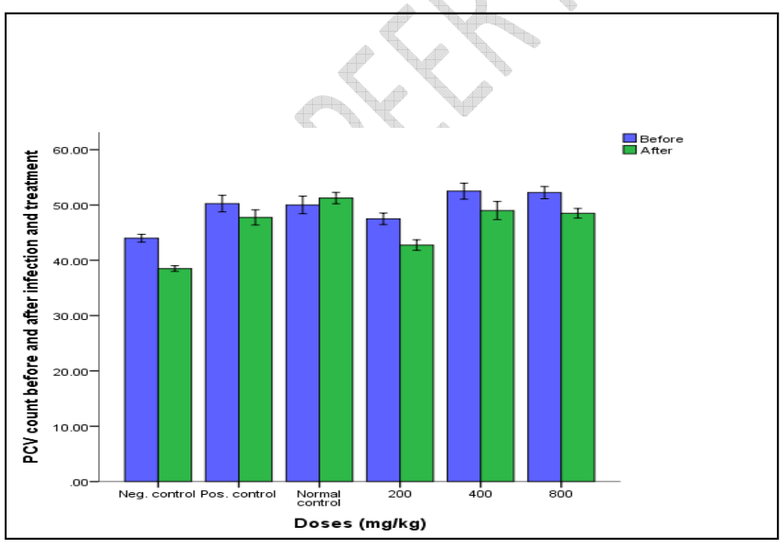

Figure 1: PCV of Mice Before and After Infection and Treatment.

Percentage Parasitemia Count.

Figures 2 shows the results of the percentage parasitaemia counts in mice of all groups. Mice in group 6 (800 mg/kg), group 2 (chloroquine treated) and group 3 (normal control) had zero parasitaemia counts for day 1, 2 and 3. However, for day 1, groups 4 and 5 (200 and 400 mg/kg of the extract) recorded low percentage parasitaemia counts compared with group 1 (infected not treated). Also, from day 2, the percentage parasitaemia counts in mice of groups 4 and 5 were significantly lower compared to mice of group 1, however

mice of group 5 (400 mg/kg) showed low counts compared with mice of group 4 (200 mg/kg). Comparative observations of groups 4 and 5 mice for day 1 and day 2 revealed a significant decrease (P<0.05) in the percentage parasitaemia counts from day1 to day2. Day 3 observations revealed a significant increase (P<0.05) in percentage parasitaemia in groups 1, 4 and 5 compared to day1 and 2. Observation from day 4 revealed a significant increase (P<0.05) in percentage parasitaemia for groups 2, 4, 5 and 6 compared to day 1, 2 and 3. After 5 days of treatment, the percentage parasitaemia counts were significantly lower (P<0.05) in mice treated with 800 mg/kg body weight of the extract compared with other extract treated groups. However, percentage parasitaemia was significantly lower (P<0.05) in mice treated with chloroquine compared to mice treated with the highest dose of the extract (800 mg/kg).

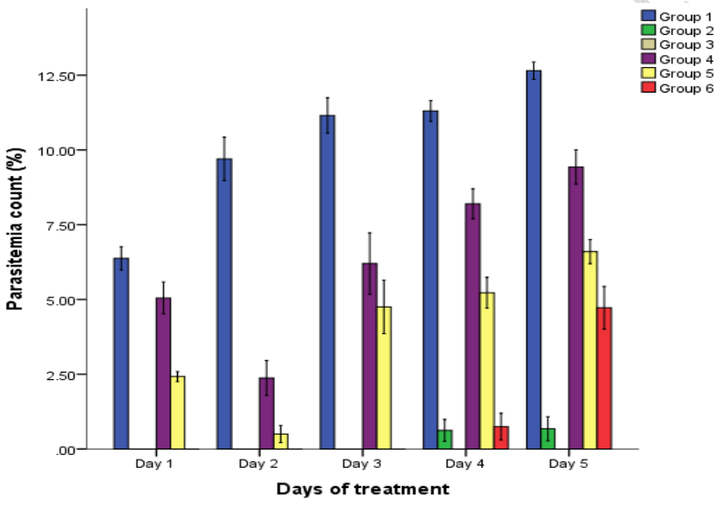

Figure 2: Parasitaemia Count (%).

Biochemical Analyses

Table 2 shows the result of biochemical parameters in mice treated with various doses of the ethanolic leaf extract of *E. ciriodora*. There was a significant difference (P<0.05) in the level of AST in mice of all groups. The AST values obtained from mice of group 1 (negative control) were higher than the observed values in groups 2, 3, 4, 5 and 6. A similar trend was also observed with ALT and Total bilirubin level. The AST, ALT and Total bilirubin level levels in mice of extract treated groups (groups 4, 5 and 6), significantly increases (P<0.05) compared to the mice of positive and normal controls (groups 2 and 3). However, the observed values of AST, ALT and Total bilirubin level in extract treated mice (group 4, 5 and 6), was lower in mice treated with 800 mg/kg body weight (group 6) compared to mice of group 4 and 5 (treated with 200 and 400 mg/kg body weight of extract). Furthermore, there is no significant difference (P>0.05) between the observed values of Total bilirubin level in mice treated with 5 mg/kg of chloroquine and 800 mg/kg of extracts (groups 2 and 6). A similar trend was also observed with the total

bilirubin level in mice of groups 4 and 5. The observed values of both BUN and Creatinine in mice of group 1 (negative control) were lower compared with groups 2, 4, 5 and 6 (chloroquine and extract treated groups) and group 3 (normal control). There was no significant difference (P>0.05) between the observed values of BUN in mice of group 3 (normal control) and group 6 (800 mg/kg of extract i.e. highest extract dose). A similar trend was observed with the BUN level in mice of groups 1 and 4 (negative control and the lowest dose of the extract i.e. 200 mg/kg). For the obtained values of creatinine, no significant difference (P>0.05) existed between groups 2 and 3 (positive and normal controls), a similar observation of creatinine values in mice of groups 4 and 5 (200 and 400 mg/kg) was recorded. The obtained levels of cholesterol and triglycerides in mice of group 1 (negative control) were higher than the observed levels in mice of groups 2, 3, 4, 5 and 6. However, in extract treated groups, the recorded levels of cholesterol and triglycerides were significantly lower in mice treated with the highest dose (800 mg/kg b. w.) The levels of cholesterol in mice of groups 3 and 5 (normal control and 400 mg/kg b. w.) showed no significant difference (P>0.05). Similar trends were observed in the levels of cholesterol between groups 2 and 6 (chloroquine and 800 mg/kg b. w.), also, a similar trend occurred between the levels of cholesterol in mice of groups 3 and 4 and, as well as groups 4 and 5. However, the levels of cholesterol in mice of groups 1 and 6 (negative control and 800 mg/kg b. w. extracts i.e. highest dose) were significantly different (P<0.05) from groups 2, 3, 4 and 5. Mice of groups 3, 5 and 6 showed no significant difference (P>0.05) in the levels of triglycerides compared to groups 1, 2 and 4. The values of HDL and LDL obtained in mice of group 1 (negative control) was significantly different (P<0.05) from the observed values in mice of groups 2, 3, 4, 5 and 6. For extract treated group, the obtained values of HDL and LDL was lower in group 6 compared to groups 4 and 5. Mice of groups 2, 3 and 6 showed no significant difference (P>0.05) in the levels of HDL, also the same observation in mice of groups 4 and 5. Similar trends of no significant existed between the LDL in mice of groups 3, 5 and 6, as well as mice of groups 2 and 4.

Table 2: Biochemical Parameters of mice

	Biochemical parameters								
Groups	AST (µl)	ALT (µl)	T.Bil (mg/dl)	BUN (mg/dl)	Creat (mg/dl)	Chol (mg/dl)	Trig (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
1	302.33±0.88^{f}	46.00±0.57^{f}	0.60±0.00^{d}	13.63±0.12^{a}	0.50±0.01^{a}	50.33±0.88^{d}	56.00±0.00^{d}	16.66±0.33^{c}	15.76±0.08^{c}
2	186.00±0.57^{b}	21.00±0.57^{b}	0.31±0.00^{b}	25.40±0.05^{c}	0.80±0.00^{d}	39.00±0.57^{ab}	45.66±0.33^{a}	13.43±0.23^{a}	13.60±0.11^{b}
3	176.66±0.88^{a}	19.00±0.57^{a}	0.20±0.00^{a}	28.00±0.57^{d}	0.80±0.00^{d}	40.00±0.57^{bc}	47.33±0.88^{ab}	14.13±0.40^{a}	12.66±0.33^{a}
4	216.66±1.20^{e}	35.00±0.57^{e}	0.41±0.00^{c}	13.50±0.17^{a}	0.60±0.00^{b}	41.66±0.33^{c}	50.00±0.57^{c}	15.66±0.33^{b}	13.66±0.33^{b}
5	199.66±0.88^{d}	29.00±0.57^{d}	0.40±0.00^{c}	18.00±0.57^{b}	0.60±0.00^{b}	40.00±0.57^{bc}	47.83±0.92^{ab}	15.33±0.33^{b}	12.20±0.15^{a}
6	191.00±0.57^{c}	25.00±0.57^{c}	0.30±0.00^{b}	27.16±0.46^{d}	0.70±0.00^{c}	38.66±0.88^{a}	46.00±0.57^{ab}	13.33±0.24^{a}	12.10±0.05^{a}

DISCUSSION

Phytochemicals screening of the ethanolic leaf extract of *E. citriodora* that contained alkaloids, saponins, cardiac glycosides, tannins, flavonoids and anthraquinone tested, agrees with Yaya *et al*. (12).

The decreased value of PCV in mice of all groups (treated and not treated groups) compared with the mice in normal control (not infected, not treated) is expected and could be due to aneamia as a result of destruction of RBC by *Plasmodium*, this agrees with the reasons advanced by Kabiru *et al*. (13), that the presence of *Plasmodium* parasites in the bloodstream results in anaemia due to active lysing of RBC. The differences in the values of the PCV for treated groups compared with control groups were concentrations independent. However, the observed PCV values in the extract treated groups compared with the normal control group were within the normal range of PVC for adult mice. This agrees with Musa (14).

The observed decrease in percentage parasitaemia in mice of group 4, 5 and 6 (extract treated groups) compared to group 1 (infected and not treated) is expected. The degree of parasite reduction was high in group 6 (800mg/kg). This significance decrease ($P<0.05$) was dose and time dependent. This agrees with the work of Kabiru *et al*. (13), who on antiplasmodial activities of the aqueous and methanol extracts of *Eucalyptus* observed a reduction in percentage parasitaemia after 5 days of treatment. Also, the zero parasitaemia counts observed respectively for the highest dose (800mg/kg) of the extract for day1, 2 and 3 of treatment can be favorably compared with the chloroquine (group 2). This is in line with similar finding by Akanbi (15), who observed that the parasite growth inhibition in positive control (chloroquine treated group) was almost similar with the group treated with highest dose of 200 mg/kg body weight of *A. leiocarpus*. This finding suggests that, *E. citriodora* leaf extract could be used in the treatment of malaria if purified. Also, the reduced percentage parasitaemia in extract treated mice could be attached to the findings of Dada and Oloruntola, (9), that alkaloids play a particular role on malaria treatment, because quinine is the first chemical that was identified in alkaloid and is used for malaria treatment.

The observed increase in the levels of AST and ALT in mice of group 1 (negative control) could be due to reasons advanced by Akanbi (15), that is an indication of *P. berghei* infection and leakage of hepatic cell that were damaged by the immune response or due to activation induced by the parasite during the hepatic stage life cycle. Also, the significant increase in value of AST and ALT in extract treated groups compared with positive and normal controls agree with Akanbi (15), who stated that the increase in AST and ALT in mice of extract treated groups might be as a result of accumulation of free radical generated by the extract used to treat the mice, which may also responsible for the destruction of the parasite. The lowest values of AST and ALT obtained in mice of group 6 (highest extract treated group i.e. 800 mg/kg bw) compared with other extract treated groups is in line with findings of Akanbi (15), who stated that, it reflects the rate of parasite clearance in that group. The reduced values of AST and ALT in mice of extract treated groups agree with Oloruntola *et al.* (16), who suggests that it could be due to hepatoprotective ability of the extract or it might be due to the relatively lower concentration or short-term administration of the extract. The increased level of total

bilirubin in mice of group 1 might be due to the breaking down of RBCs as a result of malaria infection, this is in line with the report of Oloruntola *et al.* (16). The observed insignificant difference ($P<0.05$) between the values of total bilirubin in mice of group 2, 4. 5 and 6 (chloroquine and extract treated groups) compared with group 1 and 3 (negative and normal controls) agree with Oloruntola *et al.* (16), who suggests that the leaf extract might be safe and does not pose any negative adverse effect on the bile duct or haemoglobin metabolism pathway. Urea and creatinine are the indicator of renal functions. The observed decrease in levels of urea in mice of group 1 (negative control), might be due to lower rate of urea synthesis in the liver, this agrees with Modaresi *et al*. (17). The observed increase in creatinine in extract treated groups compared with negative control mice are in line with the report of Balogun *et al*. (18), who observed an increase of creatinine in mice of extract treated groups and reported that creatinine level can be either normal or high during renal diseases. The increase levels of cholesterol in mice of group 1 (negative control) compared with other groups agree with the report of Oloruntola *et al.* (16), who attributed it to the decreased uptake of cholesterol by the infected red blood cells in high level of parasiteamia load. Several studies have showed that high plasma cholesterol, triglyceride and LDL-C are the major cause of cardiovascular disease. The significant reduction in the values of cholesterol, triglyceride and LDL-C in mice of extract treated groups suggests it uses to prevent cardiovascular infection, this is in line with findings of Momoh *et al.* (11). Also, the reduced levels of cholesterol in mice of extract treated groups might be due to the findings advanced by Lebari (19), that the leaf extract might contain saponin, that saponin is a known antinutritional factor which reduces the uptake of certain nutrients especially cholesterol at the gut through intraluminal physiochemical interactions. The observed increase in triglycerides in mice of group 1 (negative control) could be due to reasons advanced by Olusegun (20), that malaria has been implicated to cause rise in triglyceride concentration, because it is expected that the level of the triglycerides should decrease as the parasite load increases. The reduced levels of triglycerides in mice of extract treated groups compared with mice of untreated group could be an indication that *E. citriodora* extract might be used to prevent cardiovascular infections, this is in line with the findings of Momoh *et al*. (11). Also, the observed reduction in triglyceride level in mice of extract treated groups compared with mice of untreated group might be due to saponin contained in the leaf extract, saponin is a known antinutritional factor which reduces triglycerides, this agrees with Lebari (19). The observed high level of HDL-C in mice of group 1 (negative control) is not in line with the findings of Olusegun (20), who observed reduction in the HDL-C level in the negative control and explain that it could be due to the high level of parasite load in this group. The increased level of LDL-C in negative control of this study disagrees with Olusegun (20), who observed decrease in the level of LDL-C in negative control and stated that the reduction might be due to the high parasite load in the negative control. Also, the decreased level of LDL-C in mice of chloroquine and extract treated groups is not in line with the findings of Olusegun (20), who observed high level of LDL-C in mice of extract and chloroquine treated groups as a result of the decrease in the parasite load in these two groups.

CONCLUSION

The results of this investigation revealed that, the ethanolic leaf extract *of E. citriodora* exhibited the properties that may not exact toxic effect on the internal organs like liver, kidney and heart of infected treated mice. Further investigation should be carried out on the pure, active components of the leaf extract of the *E. citriodora* responsible for these actions and the effect on long term administration is recommended for further studies.

ETHICAL APPROVAL

The whole experimental management, handling and care were approved by the Research and Ethics Committee of Microbiology Department School of Science, The Federal University of Technology, Akure, Nigeria.

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