Original Research Article

ANTI-ANAEMIC AND ANTI-LEUKOPENIC EFFECTS OF HYDROMETHANOLIC EXTRACTS OF TIGER NUT AND GINGER ON ALLOXAN - INDUCED DIABETIC RATS.

ABSTRACT

Aims: This study investigated the anti-anaemic and anti- leukopenic effects of hydromethanolic (1:4) extracts of *Cyperus esculentus* (Tiger nut) and *Zingiber officinale (Ginger)* on the alloxan- induced diabetic albino rats.

Place: The*in vivo* study took place in the Department of Biochemistry, Micheal Okpara University of Agriculture Umudike, Abia state.

Methods: Fresh *Cyperus esculentus* and *Zingiber officinale* was air dried at room temperature, grounded into fine powder and extracted with hydromethanol in ratio of 20:80 using Soxhlet extraction method. The albino rats (total number of 36) were randomly divided into 6 groups of 6 rats each: Group 1 (Normal control); Group 2 (Diabetic); Group 3(Glibenclamide);Group 4 (500mg/dl *Cyperus esculentus*); Group 5 (500mg/dl *Zingiber officinale*) and Group 6 (250mg/dl *Cyperus esculentus* + 250 mg/dl *Zingiber officinale*, 50:50)were administered orally. Group 2 to 6 were administered with 160mg/kg alloxan monohydrate intraperitoneally to induce a diabetic state. The haematological parameters determined were PCV, HB, RBC, TWBC, MCV, MCH and MCHC.

Results: The diabetic group treated with the Glibenclamide and 500 mg/kg *Zingiber officinale* extract had significant recovery (compared with the normal control) from the diabetic-induced depletion of Hb, PCV and RBC than the diabetic group treated with 250 mg/kg *Cyperus esculentus* + 250 mg/kg *Zingiber officinale* extracts. 500 mg/kg *Cyperus esculentus*extract showed the least recovery from diabetic induced anaemia.

Conclusion: Ginger extract showed value in the recovery from anaemia and leucopenia associated with diabetes having significantly improved the values of RBC, PCV, haemoglobin and TWBC in the diabetic rats more than the effects of Tigernut and combined (ginger and tigernut) extracts. Ginger extract treatment might also increase the defense mechanism of the body against infections in diabetes.

Keywords: Antidiabetic, Anti-anaemic, Anti-leucopaenic, *Z. officinale, C. esculentus.*

Abbreviations: PCV- packed cell volume, HB- haemogloin, RBC- red blood cell, TWBC - total white blood cell count, MCV- mean cell volume, MCH - mean cell haemoglobin and MCHC- mean cell haemoglobin concentration.

1. INTRODUCTION

Haematological parameters are measurements that are related to the blood and blood-forming organs. There is need to monitor haematological parameters in diabetic subjects [1]. Several hematological changes affecting the red blood cells (RBCs), white blood cells (WBCs), and the coagulation factors are shown to be directly associated with Diabetes mellitus [2] other hematological abnormalities reported in the Diabetes mellitus patients include RBCs, WBCs, and platelet dysfunction [3,4]. Anaemia in diabetes mellitus may be due to the oxidative destruction of circulating red blood cells by the administered alloxan monohydrate. [5] had

reported that in diabetics, hypochromic anaemia may be due to fall in the body's iron content caused by increasing oxidation.

Cyperus esculentus (Tiger nut.) also called 'chufa sedge', is a tuber known under various names such as: nut grass, earth or ground almond, yellow nut and edible galingale. It is also called "ayaya" in Hausa, "ofio" in Yoruba [6,7] and "Aki-Hausa" in Igbo. It is commonly used as a healthy food for humans and animals in some parts of the world like Africa, Europe and America [8]. *Cyperus esculentus* have shown to contain alkaloid, saponin, flavonoid, glycoside, tannin, carbohydrates, reducing sugar and phenols[9]. Flavonoids are phenolic substances known to be synthesized by plants which possess some biological propertiessuch as anti-inflammation, anti-diabeticand cell proliferation activities. Alkaloids, saponins, and tannins are known to have antimicrobial activity, as well as other physiological activities [10,11]. *Cyperus esculentus* contains some minerals such as P, K, Ca, Fe, Zn, Mg, traces of Cu and Mn as well as some vitamins like E and C. In addition, high content of soluble glucose and unsaturated fatty acids (oleic acid and linoleic acid) [12]. The medicinal properties of *Cypserus esculentus*are rarely discussed, although its usage in orthodox activities is well known [13].

Zingiber officinale, commonly known as Ginger, is an ancient spice consumed worldwide for culinary and medicinal purposes. It is also called "Jinja" in Igbo, "Cithar" in Hausa and "Atale" in Yoruba. The plant has a number of chemicals responsible for its medicinal properties, such as anti-arthritis, anti-inflammatory, anti-oxidant, anti-diabetic, antibacterial, antifungal, anticancer, etc. [14, 15, 16, 17]. The phytochemical composition of the Z. officinale has been extensively studied in the past studies. Z. officinale is reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, saponins, steroids, terpenoids and tannin as the major phytochemical groups. These phytochemicals play an important role in the medicinal property of this plant [18, 19].

This study focused to determine and compare the anti-anaemic and anti-leucopaenic potentials of *Cyperus esculentus* (Tiger nut) and *Zingiber officinale* (Ginger) extracts in alloxan-induced diabetic wistar rats.

2. MATERIALS AND METHODS

2.1 Plants

Fresh *Cyperus esculentus* and *Zingiber officinale* was obtained from Ekeonunwa, Owerri municipal in Imo state, Nigeria and identified at the Botany Department, Micheal Okpara University of Agriculture Umudike.

2.2 Chemicals

Chemicals used in this study included Methanol (extra pure) Manufactured by Loba Chemiepvt. Ltd. 107 Wodehouse road, Mumbai 400005, India, Alloxan monohydrate Manufactured by Qualikems fine chem pvt. Ltd. Plot no. 68/69, G.I.D.C. Industrial estate, Nandesari, Vadodara, Gujarat. Glibenclamide Manufactured by Nigeria German Chemicals Plc. Km 38 Abeokuta express way, Otta, Ogun state, Nigeria.

All chemicals were purchased from Body Scientific Chemicals Aba Nigeria.

2.3 Equipment

The equipment used were obtained from the Department of Biochemistry, Micheal Okpara university of Agriculture Umudike, Abia state.

They include thermometer, micropipette (perfect,USA), refrigerator (Haier thermocool, England), spectrophotometer, syringe, water-bath, weighing balance, petridish, glass ware, beakers, capillary tube, micro-haematocrit centrifuge,soxlet extractor, hemocytometer, test tubes, gavage, glucometer(Acucheck), glucose stripes.

2.4 Preparation of Extracts

Extracted liquid was obtained from the grounded air-dried *Cyperus esculentus* and *Zingiber officinale* using the Soxhlet extractor as adapted from [20]. They were extracted with 350ml of hydromethanol which consists of 20% water and 80% of methanol. After which was put in the oven for 40^{o}C and allowed to evaporate to form a semi Solid.

2.5 Animals

The experimental animals use for the study were 36 Wistar albino rats (*Rattus norvegicus*)within the weight of (90-117g). The rats were obtained from Animal holding unit of the Department of Biochemistry, Micheal Okpara University of Agriculture Umudike. The animals were kept in a well-ventilated and clean cages at an average room temperature of 30^{o}C, the rats were fed with cubes produced by Livestock Feeds PLC and bought from the local shop and water ad libitum which was changed daily. The rats were allowed to acclimatize to the new environment for 7 days.

The toxicity study was also carried out with additional 36 albino Wistar rats.

2.5.1 Alloxan-induced diabetic animal model

Albino wistar rats weighed (90-117g) after fasting for 24hrs. Animals in the diabetic group were subjected to a single intraperitoneal injection of alloxan monohydrate at 160 mg/kg body weight [21], freshly dissolved in sterile distilled water. The rats were given 5% glucose solution after 12 hours of alloxan injection to drink overnight to counter hypoglycemic shock. 4 days after alloxan injection, fasting blood glucose (FBG) was determined using glucometer with model (Accu-chek active, Roche Diagnostic). Rats showing FBG above 200 mg/dl were considered diabetic, as described by [22].

2.5.2 Administration of Alloxan

1g of alloxan dissolved in 20 mls of H_2O ⟶ 1g/20ml = 0.05g/ml ×1000 =50mg/ml

For every animal weighed (dose administered) = Alloxan dosage (mg) X body weight(g) /Concentration X 1000
160mg/Kg X 117g/50mg/ml X 1000 = 0.37ml
Therefore, each rat was given 0.4ml of Alloxan.

2.6 Acute toxicity study/ Determination of Median Lethal Dose (LD_{50})

This was done using Lorke method [23]. A total of 36wistar rats (18 rats each for *Cyperus esculentus* and *Zingiber officinale*) were used for the study. The study was carried out in two phases for each extract (*Cyperus esculentus* and *Zingiber officinale*). In the first phase,

three groups; A-C of 3 rats each were orally gavaged with distilled water (10 ml/kg), 10 mg/kg, 100 mg/kg and 1000mg/kg (lower doses) of the tiger nuts extract respectively. Rats in group A served as the normal control. The rats were observed for signs and symptoms of toxicity and mortality over a period of 24 hours. The same procedure was repeated with the ginger extract.

In the second phase, three groups;with 3 rats in each group, were given (orally) distilled water, 1600 mg/kg, 2900 mg/kg and 5000mg/kg (higher doses) of the extracts respectively. Rats in Group 1 served as the normal control. The rats were observed for 24 hours, post administration. The same procedure was followed with the ginger extract.

In each phase of the acute study, the rats were allowed for another 7 days to observe any delayed toxicity. The median lethal dose (LD_{50}) of the two extracts were then calculated using the formula adopted by [24].

$$LD50 = \sqrt{(\text{Least dose with mortality} \times \text{Highest dose without mortality})}$$

2.7 Experimental Design

The rats were divided into 6 groups of six rats each: Group 1: Normal control rats were administered with 1 ml of distilled water once daily for 14 days. Group 2: Diabetic control rats were administered with 1 ml of distilled water once daily for 14 days. Group 3: Diabetic rats were treated with *Cypserus esculentus* extract (500 mg/kg body weight/day) for 14 days. Group 4: Diabetic rats were treated with *Zingiber officinale* extract (500 mg/kg body weight/day) for 14days. Group 5: Diabetic rats were treated with both*Cypserus esculentus* and *Zingiber officinale* extract (50:50) (500 + 500mg/kg) for 14 days. Group 6: Diabetic rats were treated with Glibenclamide (5 mg/kg body weight/day)for 14 days.

Extracts of tiger nut, ginger and Glibenclamide was administered to the experimental albino rats based on design groups through oropharyngeal cannula for 14days. Their respective body weight sample was taken before and after treatment of oral administered of drugs**.**

2.6 Collection of blood samples

Blood samples were taken from each rat by terminal bleeding from the heart and transferred into a clean EDTA container (thoroughly mixed) ready for haematological investigations.

2.7 Evaluation of Haematological Parameters

The Haematological parameters evaluatedfollowing (25) were: haematocrit (Packed cell volume), Haemoglobin Concentration, Red Blood Cell Count,Total White Blood Cell Count, Mean cell volume, Mean cell haemoglobin, and Mean cell haemoglobin concentration.

2.8 Statistical Analysis

All the data were tested for normally using Mann-Whitney's normality test. Statistically comparisons were performed for haematology and glucose levels using one-way analysis of variance (ANOVA) followed by Duncan's multiple range tests to separate the mean. The results were presented in tables and expresses as mean ± SEM and p value less than 0.05 ($p<0.05$) was considered statistically significant. The data were analyzed using SPSS version 23.0.

3. Results

Table 1 represent the acute toxicity test of *Cypserus esculentus* and*Zingiber officinale*on albino wistar rats at different doses. The acute toxicity test of *Cyperus esculentus and Zingiber officinale*extracts showed no death in the both phases of the experiment at different doses.

Table 1 Acute toxicity test of *Cypserus esculentus (C.E)and Zingiber officinale (Z.O)*

Phase 1	Number of rats	Dosages mg/kg body weight	Mortality of C.E	Mortality of Z.O
Group A	3	10mg/kg	0/3	0/3
Group B	3	100mg/kg	0/3	0/3
Gro0up C	3	1000mg/kg	0/3	0/3
Phase 2				
Group A	3	1600mg/kg	0/3	0/3
Group B	3	2900mg/kg	0/3	0/3
Group C	3	5000mg/kg	0/3	0/3

Table 2: Haematological data obtained from the Control Groups and after 14-day treatments with*Cyperus esculentus* and *Zingiber officinale*extracts of alloxan induced diabetic rats.

Animal Groups	**Hb(g/dL)**	**PCV (%)**	**RBC ($\times10^6$mm)**	**TWBC ($\times10^3$/mm^3)**	**MCV (fL)**	**MCH (pg)**	**MCHC (g/dL)**
Normal control	14.72±0.26^a	38.60±1.50^a	6.09±0.26^a	7.85±0.85^c	66.71±0.49^b	24.26±0.65^a	38.26±0.84^b
Negative control	7.84± 0.62^c	20.40±0.81^c	3.25±0.14^c	15.57±0.88^a	69.06±0.60^a	23.94±0.93^b	38.24±1.62^b
Std. control	13.72±0.25^a	34.00±0.54^a	5.45±0.07^a	9.97±0.50^c	65.96±0.20^b	25.17±0.20^a	40.35±0.30^a
500mg/kg C.E	10.56±0.29^b	27.60±1.16^b	4.42±0.16^b	12.10±0.77^a	66.90±0.33^b	23.95±0.80^a	38.44±1.48^b
500mg/kg *(Z.O)*	13.28±0.88^a	32.40±2.61^a	5.21±0.41^a	8.24±0.65^c	66.29±0.51^b	25.57±0.58^a	41.19±0.96^a
500mg/kg (C.E+Z.O)	12.64±0.41^a	32.20±1.01^a	5.09±0.17^a	9.67±0.94^b	66.85±0.40^b	24.81±0.44^a	39.26±0.53^b

(NOTE: Cyperus esculentus (C.E) Zingiber officinale (Z.O). standard (std) control *(*Glibenclamide*).* Values are presented as mean ± Standard Error of Mean. Means compared along columns with different superscript signifying significantly different at $p<0.05$, superscript a, b, c and d signifies mean differences, where values with letter a means that it was significantly higher than the value with superscript b while b was significantly higher than superscript c and c is significantly higher than superscript d. RBC (Red Blood Cell); PCV (Packed Cell Volume); Hb (Haemoglobin); TWBC (Total

White Blood Cell); MCV (Mean Corpuscular Volume); MCH (Mean Corpuscular Haemoglobin) and MCHC (Mean Corpuscular Haemoglobin Concentration).

4. Discussion

Most of the natural plant extracts used in traditional medicine are believed to be safe, compared to synthetic drugs, even when there are no toxicological records or scientific evidence to this believe [26], hence the need to carry out the acute toxicological studies on the two extracts (tiger nut and ginger) using rats.

As shown in Table 1, after 24 hours post administration of the tigernut and ginger extracts, at both high and low doses no death was recorded in all the extract groups and the distilled water group. Therefore, the result of the LD_{50} of the two extracts were estimated to be $\geq$ 5000mg/kg body weight in mouse which is a likely indication that the two extracts are non-toxic. It is important to recall that the organization of Economic Cooperation and Development (OECD), Paris, recommended LD_{50} value < 50mg/kg as very toxic; > 50 $\leq$ 500 mg/kg as harmful; > 500 $\leq$ 2000 mg/kg as not harmful or toxic; and $\geq$ 5000 mg/kg body weight as very safe [27]. Also, according to [28,29] who reported that substances with an LD_{50}> 1000 mg/kg are of low toxicity or are relatively safe. Hence, the two extracts; tigernut and ginger with an $LD_{50} \geq 5000$ mg/kg can be said to have very high safety margin.

The result presented in Fig. 1 showed that the Hb concentration, PCV and RBC in the diabetic untreated rats were significantly ($p<0.05$) reduced from 14.72g/dL, 38.60%, 6.09×10^6/mm^3 (normal control) to 7.84g/dL, 20.40%, 3.25×10^6/mm^3, respectively. Whereas, the diabetic group treated with 500mg/kg CE showed significant recovery by an average of 10.52g/dL (Hb), 27.60% (PCV), 4.42 $\times10^6$/mm^3 (RBC) and Gilbenclamide (standard drug) showed significant recovery by of 13.72g/dL (Hb), 34.00% (PCV), 5.45 x 10/mm^3 (RBC), (compared with the normal control).

From the results on the effects of the extract on the haematological values of the diabetic rats presented in Fig. 1, it was observed that haematogical parameters including RBC, PCV and Hb were significantly low in the diabetic untreated rats when compared with the normal control group. This suggest that the diabetic agent used in this study potentially caused significant level of aneamia, and the extent of recovery observed in the treated groups showed that 500 mg/kg tigernut extract behaved as a potent anti-anaemic agents much like the standard antidiabetic (Glibenclamide) drug in diabetes.

Patients with diabetes suffer the consequences of impaired renal function earlier in the course of their disease than do their non diabetic counterparts [30]. In diabetic nephropathy (DN), anemia tends to be more severe than in non-diabetic renal disease and occurs at an earlier stage of the disease. Anaemia in diabetes mellitus may be due to the oxidative destruction of circulating red blood cells by the administered alloxan monohydrate. [31] had reported that in diabetics, hypochromic anaemia may be due to fall in the body's iron content due to increasing oxidation.

The induction of diabetes mellitus did not significantly alter the mean values of MCH and MCHC, but caused significant ($p<0.05$) increasein the mean value (69.06fL) of MCV (Mean Corpuscular Volume) in the diabetic untreated group compared with an average value of 66.90Fl (tiger nut extract) and 65.96Fl (Standard drug) obtained in the treated groups, suggest progressive return from macrocytic cells (which is characteristics of haemolysis or excessive loss of blood, and corresponding effect via compensatory mechanism by the haemopoetic tissues) to normocytic cells by the treatment extracts. The mean value of MCV in Gilbenclamide treated group was statistically similar with the mean value obtained in the 500 mg/kg tigernut extract treated group. This effect might be due to the phytochemical content of tiger nut as reported by [32].

Results obtained for 500 mg/kg tiger nut in this study is consistent with the findings of [33], who reported that tiger nut extract significantly increased the values of RBC, PCV and Hb in a dose dependent manner in rats, although the values of MCV and MCHC were not significantly altered in that study. This positive effect of tiger nut on RBC values also corroborates with the findings of [34], who reported significant increase in RBC, Hb and PCV values in rats following treatment with tiger nut extract.

4.3. Effect of 500 mg/kg Ginger on the haematological parameters of alloxan induced diabetic rat.

The results presented in Table Fig. 2 showed that the Hb concentration, percentage mean PCV and the average number of RBC in the diabetic untreated rats were significantly ($p<0.05$) reduced from 14.72g/dL, 38.60%, $6.09\times 10^6/mm^3$ (normal control) to 7.84g/dL, 20.40%, $3.25\times 10^6/mm^3$ respectively. Whereas, the diabetic group treated with the 500mg/dl Ginger showed significant recovery, 13.28g/dL, 32.40%, $5.21\times 10^6/mm^3$ respectively (compared with the diabetic, 500mg/dl tiger nut and combined extracts).

In this experiment, increased RBC count of Z. officinale–treated rats, when compared to the negative control, could be due to the lowered lipid peroxide level in RBC membrane leading to a decreased susceptibility of RBCs to haemolysis. Because non-enzymatic glycosylations of membrane proteins correlate with hyperglycemia [35], it might be said that Z. officinale produced its effect by decreasing the elevated glucose concentration in Z. officinale–treated rats.

Total White blood cells (TWBC) are involved in body defense against infection [36]. It has been suggested that the body's defense mechanism against infection is disturbed due to the disturbed WBC function in diabetes [37]. In this experiment, we demonstrated that Z. officinale increased the lowered WBC to control levels. This result indicates that Z. officinale treatment might also increase the defense mechanism of the body against infections in diabetic rats.

The induction with the diabetic agent did not significantly alter the mean values of MCH (Mean Corpuscular Haemoglobin), MCHC (Mean Corpuscular Haemoglobin Concentration) implying normal Corpuscular Haemoglobin Concentration, but caused significant ($p<0.05$) increases in the mean value (69.06fL) of MCV (Mean Corpuscular Volume) in the diabetic untreated group, compared with an average value of 65.96fL (standard drug) and 66.29fL (ginger extract) obtained in the treated groups, suggest progressive return from macrocytic cells

(which is characteristics of haemolysis or excessive loss of blood, and corresponding effect via compensatory mechanism by the haemopoetic tissues) to normocytic cells by the treatment extracts. The mean value of MCV in Gilbenclamide treated group was statistically similar with the mean value obtained in the 500 mg/kg ginger extract treated group.

The result of the TWBC showed significant increase from normal mean value of 7.85 $\times 10^3/mm^3$ to 15.57$\times 10^6/mm^3$ in the diabetic untreated group compared with 500 mg/kg ginger extract treated groups, 8.24 $\times 10^3/mm^3$, whose mean TWBC value was not significant ($p > 0.05$) and almost the normal range. The mean value of TWBC in Gilbenclamide treated group was statistically the same ($p > 0.05$) with the mean value obtained in the 500 mg/kg ginger extract treated group.

This study is consistent with the findings of [38] who reported significant changes in RBC and WBC values in rats following treatment with Ginger extract.

4.3 Effect of 500 mg/kg Tiger nut and 500 mg/kg Ginger on the haematological parameters of alloxan induced diabetic rat.

The results showed that the Hb concentration, percentage mean PCV and the average number of RBC in the diabetic untreated rats were significantly ($p<0.05$) reduced from 14.72g/dL, 38.60%, 6.09$\times 10^6/mm^3$ (normal control) to 7.84g/dL, 20.40%, 3.25$\times 10^6/mm^3$ respectively. Whereas, the diabetic group treated with combined 500mg/kg extracts showed significant recovery to an average of 12.64g/dl(Hb), 32.20%(PCV) and $5.09\times 10^6/mm^3$ while Gilbenclamide showed significant recovery (compared with the normal control). This showed that both extracts significantly improved haematological values in the diabetic rats. This effect of the extract may be due to their iron content.
The role of iron in haemoglobin and red blood cells formation is well established [39]). Iron in green leafy vegetables is reportedly responsible for the haematinic effects of such plants [40]. The positive effects exerted by tiger nut and ginger on the haematological values of diabetic may also be due to their reported antioxidant effects [41,42]. Antioxidant agents are known to be major actors in reversing the haematological anomalies associated with diabetes mellitus [43]. Another likely mechanism for the observed effects may be that the extracts enhanced the synthesis of erythropoietin, a hormone that stimulates the production of RBCs in the bone marrow initially disrupted by the administration of alloxan.

The result of the TWBC showed significant increase following treatment with the extracts, suggesting that the extracts could enhance the responsiveness of the rat immune system indicating an immune involvement in the resultant anaemia. Furthermore, the rapid rise in TWBC mean values following the chemical induction of diabetes mellitus may be due to the activation of a secondary immune response via activation of B lymphocytes thereby inducing an amnestic response and secondly, induced membrane damage rendering erythrocytes susceptible to recognition and destruction by macrophages. This observed WBC anomaly was however ameliorated at the end of the treatment period with tiger nut and ginger extracts. Improvements in WBC count in rats treated with these extracts have been reported [25,29].

This study suggested that the diabetic agent (alloxan) used in this study potentially caused

significant level of aneamia, and the extent of recovery observed in the treated group (500 mg/kg tigernut + 500 mg/kg ginger extract) showed that the combined extract behaved as a potent antidiabetic agent much like the standard antidiabetic (Gilbenclamide) drug. The Group treated with the combined extract of 500 mg/kg tigernut extract and 500 mg/kg ginger extract gave better activity than 500 mg/kg tigernut extract, suggesting possible synergistic antidiabetic activity. These maybe why extracts from tiger nut and ginger have over the years been used in traditional medicine [44].

5. Conclusion

Cyperus esculentus (tigernut) and *Zingiber officinale* (ginger) extracts could be safe when used orally in the management of anaemia and leucopenia associated with diabetes mellitus having significantly improved the values of RBC, PCV, haemoglobin and total white blood cells in the experimental diabetic rats. This study supports the use of these extracts in traditional medicine for the management of diabetes and its associated complications. However, further studies are required with the plant extracts. Such studies should include measurement of RBC fragility and serum folic acid, iron, cobalt, vitamin B12, calcium levels and renal functions to demonstrate the exact mechanism of actions of these extracts on increased RBC count of diabetic rats. Z. officinale extract treatment might also increase the defense mechanism of the body against infections in diabetic rats.

ETHICAL APPROVAL:

Ethical approval was obtained from the Ethical Committee of the School of Health Technology, Federal University of Technology Owerri, Nigeria.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. 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.

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