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Effects of a formulated herbal mixture from *Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B. in honey on weight, biochemical profile and hematological indices of apparently healthy Wister rats

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Abstract

Plants and plant derived products have been a source of alternate therapy for ages. This is because of availability and effectiveness in managing a wide range of illnesses. This study was carried out to assess the effects of a formulated herbal mixture made from *Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B. and honey (STBH) on weight, biochemical parameters, hematological profile and histological effect on key organs of apparently healthy Wistar rats. The plant materials and honey used were sourced locally. The herbal mixture (STBH) was formulated from *Sorghum bicolor* L., (7.74g/kg body weight), Tumeric (2.4 g/kg body weight), Honey (2.5 g/kg body weight) and *Bridelia ferruginea* (0.4 g/kg body weight) to form 13 g/kg body weight (1.3 mg/g) of the mixture. A total of 40, apparently healthy male Wistar rats, 6-8weeks old with average initial body weight of 75 g was used. The rats were randomly grouped into four groups. The first 3 groups A, B and C (n=12 each) were orally administered the herbal mixture at dosage of 0.65mg/g, 1.3mg/g and 2.6mg/g of rat weight respectively for 28 days in order to determine the safe dose while the last group D (n=4) as control. At the end of every week, the weights of the rats was measured, while the blood samples of 3 rats per treatment group and one from control group which were randomly selected were subjected to Biochemical and Haematological assays and their organs (Kidney, Heart and Liver) were harvested for histological examination following standard methods. The results revealed weight gain among the rat fed with STBH compared to the control rats with group A having the highest percentage weight gain (50.55%). Haematological results showed significant ($p<0.05$) improvement in pack cell volume (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular volume (MCV) of the rats. Moreover, significant ($p<0.05$) immunostimulatory effect was observed in group A rats compared to groups B, C and D rats. AST and ALT were considerably lower in group A rats when compared to the control, unlike the increase in values at higher dosages (groups B and C rats) though the increase was not significant ($p>0.05$) . Urea level was fairly high in groups B and C rats, while it reduced in group A rats as compared to the control group. Histology of the liver, heart and kidney of the rats fed with STBH showed near normal architecture with no significant deviations from the control. These suggest that STBH is safe, improves PCV, has immunostimulatory potential and also shows ability to maintain the normal architecture of the liver, kidney and the heart.

Keywords: Formulated Herbal mixture; Biochemical; Haematological; Histological Immunostimulatory

1. Introduction

Herbal medicine is the use of plants and plant extracts to treat diseases. Herbal products have been used by humans for thousands years to treat various ailments or to improve physical performance. Plants have always been a major source

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of nutrition and health care for both humans and animals [1]. In recent years, there has been growing interest in alternative therapies and the therapeutic use of natural products, especially those derived from plants. Despite the move towards synthetic medicine and use of sophisticated drugs, traditional plant-based remedies still play an important role in the world's medicine [2]. It is estimated that 80% of the populations of developing countries rely on traditional medicines, mostly plant drugs, for their primary health care needs [3,4]. Therefore there are considerable economic benefits in the development of indigenous medicine and in the use of medicinal plants for the treatment of various diseases. In Nigeria today, there is an upsurge in the acceptance and utilization of these herbal medicine partly because of scientific support for some of their medicinal uses, and because of the belief that "Green medicine" is cheap, safe, more dependable and accessible than the costly conventional medicine [5]. The bioactive constituents present in most plants such as flavonoids, polyphenols, alkaloids, terpenoids, saponins, enzymes, and tannins have been reported to be responsible for their effectiveness and therefore are widely used for the treatment of many diseases [6]. However, the major problems of using herbal products are the issue of dosaging and biosafety [7].

Sorghum bicolor L., *Curcuma Longa* L*. and Bridelia ferruginea* B. are important medicinal plants that are widely used in Sub-Sahara Africa [8]. However, *Bridelia ferruginea* appears to have been the most studied. *B. ferruginea* is a small non-laticiferous scaly tree or shrub that grows to about 4 meters high. The plant often bears pines and may be slash crimson coloured. The leaves may be small to medium sized, simple, alternate, spiral or distichous, broadly elliptic and pubescent, it is about 6 - 15 m high, up to 1.5 m in girth. They are also pinnately veined with entire margin and an acuminate or acute apex [8,9]. It has been shown to possess antimicrobial properties, laxative effect, anti-diabetic, anti-oxidant and anti-inflammatory properties [10, 11, 12] and inhibit Iron (II) Sulphate [13].

Curcuma longa L., is a perennial herb and member of the Zingiberaceae (ginger) family which is well known as turmeric and is cultivated extensively in Asia, India, China, and other countries with a tropical climate [14]. Turmeric has been used extensively in traditional Chinese and Ayurvedic medicinal systems because of its bioactive constituent [15]. Curcumin which is the main active constituent of *Curcuma longa,* a yellow substance has been displayed to have a wide scope of restorative impacts like hepatoprotective activity in hepatotoxicity [15, 16]. One of the major mechanisms underlying *Curcuma longa* disease-modifying effects is its pleiotropic anti-oxidant activity. It scavenges and prevents formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [15].

Sorghum is a major food crop in Africa and India and an important livestock feed in the Americas. Europe and Japan. It is known under a variety of names such as Kaffir corn in Sour Africa, guinea corn in West Africa. It is also called Karan dafi by the Hausas of Northern Nigeria and Oka Pupa (red corn) by the Yorubas of Southern Nigeria [17]. It has a variety of uses including its being the staple food for large populations in Africa and Asia. It is also used in making beers. In West Africa, dye extracted from the plant is used in coloring leather or colouring of cloths, calabashes and as a body pigment [17]. Researchers have reported S*orghum bicolor* leaf sheaths as possessing anti-inflammatory and immune-modulating properties [18], improve increased hemoglobin and CD4+ T-lymphocyte counts in HIV positive patients [19], management of anaemia and maintain kidney integrity [20]. According to Temileye *et al*. [21] *Sorghum bicolor* extract is rich in natural antioxidants that can scavenge free radicals and fight against degenerative diseases (cyclophosphamide- induced oxidative stress) thereby improving the body's antioxidant status. This is due to the presence of phytochemicals which can ameliorate free radicals and associated lipid peroxidation and cell damage.

Honey is a product made by bees using nectar from flowers. It is made up of a complex mixture of carbohydrates, proteins, enzymes (invertase, glucose oxidase, catalase, and phosphatases), amino and organic acids (gluconic acid, acetic acid, etc.), lipids, vitamins (ascorbic acid, niacin, pyridoxine, etc.), volatile chemicals, phenolic acids, flavonoids, and carotenoid-like substances and minerals which may function as antioxidants [22]. Honey has various medicinal properties, which include antibacterial, antihypertensive, hepatoprotective, hypoglycemic and antioxidant effects [23, 24, 25]. Studies have shown that honey exerts a hypoglycemic effect and ameliorates oxidative stress in streptozotocin-induced diabetic rats [26]. Ahmad *et al.,* [27] reported that honey has high anti-inflammatory potential and tissue wounds healing potential. It has also been shown to improve hemoglobin and red blood cell, increase in white blood cell and mean corpuscular hemoglobin (MCH), MCV and MCHC in rats [24].

Sorghum bicolor L., *Curcuma longa* L, *Bridelia ferruginea* B and honey have been studied by many researchers to evaluate their individual health benefits but no data is available on their biosafety especially when formulated into a herbal mixture to improve general health. Thus this study is geared towards evaluating the effects of a formulated herbal mixture from *Sorghum bicolor* L., *Curcuma longa* L, *Bridelia ferruginea* B. and honey on some hematological indices, biochemical indices and key organs (such as heart, kidney and liver) of apparently healthy rats.

2. Material and methods

2.1. Preparation of herbal mixture

The plants materials (*Sorghum bicolor* L*,, Curcuma longa* , *Bridelia ferruginea*) and honey were purchased from local market in Ado Ekiti. Plant identification was done at the herbarium at the School of Agriculture, Federal University of Technology, Akure, Nigeria. The plant materials were rinsed under running water and air dried to a constant weight before pulverized into powder with electric blender. Mixing ratio was determined according to Akande *et al.* [17], Haddad *et al.* [24] and Olarewaju *et al.* [28]. The composition of the herbal mixture is as follows:

Sorghum bicolor=7.7.4g/kg
Curcuma Longa=2.4g/kg
Honey= 2.5g/kg
Bridelia ferruginea= 0.4g/kg
Total= 13g/kg

2.2. Experimental Design

A total of 40 apparently healthy male Wistar rats, 6-8weeks old with average initial body weight of 75g were bought from College of Medicine, Ekiti State University, Ado Ekiti. The rats were acclimatized for 1 week at the animal house of Microbiology Department, Federal University of Technology, Akure and were randomly grouped into four groups. The first 3 groups A, B and C (n=12 each) were administered the herbal mixture at dosage of 0.65mg/g, 1.3mg/g and 2.6mg/g of rat weight respectively for 28 days in order to determine the safe dose while the last group D (n=4) which was used as control was left untreated. At the end of every week, the blood samples of 3 rats per treatment group and one from the control group which were randomly selected was collected and subjected to Haematological assay while their organs (Kidney, Heart and Liver) were harvested for histological examination. Organ harvesting and blood sample collection were done according to National Central for Replacement Refinement Reduction of Animals in Research [29].

2.3. Animal assay

2.3.1. Body weight measurement

Body weight of all the experimental animals was taken by using digital electronic balance before commencing the first oral administration of the herbal mixture and then weekly till last day of oral administration of the herbal mixture.

2.3.2. Sacrifice of animals and collection of blood and organs

The randomly selected rats at the end of every week were anesthetized with chloroform vapour, then placed in a supine position and the four limbs were immobilized. A dissection was performed on the rats and 5ml blood was collected by cardiac puncture. Two milliliters of the blood collected was introduced into a tube containing an anticoagulant (EDTA) for hematological analysis, the remaining 3ml was used for biochemical assays. Then, the heart, kidneys, and liver were removed and stored in 10% buffered formalin for histopathological analyzes [30].

2.4. Blood samples

Haematological parameters (Full blood count) were analyzed using 5 part haematology auto-analyzer (Model XN-L 350 Sysmex) while biochemical analysis were carried out according to Debelo *et al*. [7]

2.5. Histopathological examination

The collected organs for histopathology examination were first fixed in 10% neutral buffer formalin, dried using ethanol (70–100%), then in xylene, and lastly fixed in paraffin. A micron cut of the specimens was stained with (H & E stain) and viewed under microscope for any defect according to Olaiya *et at*. [31]. Photomicrograph of selected slides from both the treated and control groups were taken under a magnification of x40 and x20 objectives by using (EVOS XI, China) automated built-in digital photo camera.

2.6. Statistical analysis

Two-way repeated analysis of variance (ANOVA) was used. The generated data were analyzed using IBM SPSS, version 22. Bonferroni post hoc test multiple variety test was run to compare variances within means. Level of significance was tested at $p < 0.05$ (IBM 2020).

3. Results

3.1. Effects of administration of STBH on weight of the rats

There was progressive and statistically significant ($p<0.05$) weight gain across the groups. Comparing the percentage of weight gain of the groups at the end of 28 days of administration of the herbal mixture, Group A (50.55%) has the highest percentage weight gain followed by group B (43.81%), group C (37.77%), and the least weight gain was observed in the control group (36.68%) (table 1). This shows that the herbal mixture has effect on the weight of the experimental rat group as compared to the control group.

3.2. Effects of administration of STBH on PCV

The main effect of STBH was statistically significantly difference in the PCV Level for the four groups $f(3,7)= 248.348$, $p=0.00$ (Table 2). The magnitude of the difference in the mean and the effect size was large (partial eta square=0.991). Statistical power was adequate and equate to 1.00. The 1st week of the administration shows no statistically significant difference in mean among all the groups ($p>0.05$). At the second week PCV level show no significant difference between control group and group A and B ($p=1.00$, and 0.798 respectively). But group C was statistically differ from the control ($p<0.05$). This was same in 3rd week but significant difference was observed in week 4 between control and group A and B ($p=0.00$ and $p=0.001$), group C did not differed from control ($p=0.432$).

3.3. Effects of administration of STBH on RBC

Red blood cell level among the four groups were statistically significantly different $F(3,7)= 47.900$, $p=0.00$. The magnitude of the difference in the mean and the effect size was very small (partial eta square=0.954). Statistical power was adequate and equate to 1.00 (table 2). Estimated mean show that group A, B differed significantly from control group ($p=0.00$ and $P=0.003$ respectively), while group C shows no significant difference from control ($p=0.12$) (Table 3).

3.4. Effects of administration of STBH on Hemoglobin level

Difference observe red in the Hemoglobin (HGB) level of the rat among the four groups in (table 3) were statistically difference $F(3,7)= 250.088$, $p=0.00$. The administration of the herbal mixture significantly increase the HGB of the rats in group A as compared to the control, B and C($p<0.05$) while no significant difference in mean was observed group C compared to control ($p> 0.05$).

3.5. Effects of administration of STBH on MCV

The Mean Cell Volume shows no statistically significant difference in mean among the four groups and the groups fed with STHB were not significantly higher that MCV of the control group. In table 6 the Mean Corpuscular Hemoglobin (MCH) value of group A, B and C shows no significant deviation from the control group ($p>0.05$). Although group C had the decline in MCH as compared to the control. The increase observed in Mean Corpuscular Hemoglobin Concentration (MCHC ($p=0.693$)) among the groups was not statistical significant although group A MCV was fairly high compare to the control group ($p<0.05$) (Table 7).

3.6. Effects of administration of STBH on Immunocytes

White blood cells are the immune of the body system whose role is to defend the body system against invasion by pathogenic organisms such as bacteria, fungi, viruses and other foreign bodies. Decrease in WBC may signal a weak immune system. In this present study the WBC and Lymphocytes level of the rats fed with the STBH had increased mean value as compared to the control (Tables 8 and 9). This difference in mean value among the four groups were statistically significant $p<0.0002$. At the 3rd and 4th week, there was a decline in the WBC and Lymphocyte counts of all the groups to a fairly constant level but group A, B and C were still significantly higher as compared to the control group D ($P<0.0002$). This could signify body system adjustment to STBH.

The monocytes level were not significantly different between group A and the control ($p=0.072$) while groups B and C differ in mean value as compared to the control group ($p =0.048$ and $p=0.002$ respectively). The mean differences among groups followed a linear trend across the time $F(3,7)=12.959$; $p=0.003$, Partial Eta Squared=0.846, observe power=0.98 (table 10).

Granulocytes (GRAN) make up about two-thirds of your white blood cells. The mean level of granulocytes observed was statistically significantly different among the four groups, $f(3,7)= 0.734$, $p<0.0002$. The rats in groups A and B show no

significant deviation in mean from the control group (p=0.07 and p=0.678) while group C differs significantly in GRAN level as compared to control group (p=0.002) (table 11). The trend in mean across the groups was not statistically significant over time F(3,7)=14.613. The control group (1.67±0.00) at the 4th week has the highest GRAN as compared to group A (0.87±0.106), group B (0.86±0.18) and group C (0.88±0.13) (p=0.002 at Cl=95%). Eosinophil level was fairly high among the rats fed with STBH. Group B fed with 1.3mg/g dosage showed an elevated level of eosinophil.

3.7. Effects of administration of STBH on Biochemical Indices

The effect of the administration of STHB on ALT level among the different groups was statistically significant (F(3,7)=197.386, p<0.0005). Group A showed comparatively no significant difference in mean from the control group over the feeding period while group B and C had significant increase in ALT level as compared to the control. The mean level of Aspartate Aminotransferase (AST) and Creatinine (CREA) fairly increase in the rat fed with the herbal mixture in 3rd and 4th weeks as compared the control (Tables 13-15).

The Urea Level of all the groups were high right from the week one and the increase over the experimentation period was not significant. Group A (0.65mg/g dosage) had the lowest urea level follow by the control. While group C(2.6mg/g dosage) had the highest urea value (table 16).

Bilirubin in group A recorded lowest level of bilirubin in the 1st week when compare with group B, C and control group but the difference in mean was not statistical significant with group B(0.05413±0. 00153; p=0.413). In the second week group B (0.05637±0.00274) has the lowest bilirubin level but not statistically differed from bilirubin level in group D. In the third week of administration of the herbal mixture, increase in bilirubin level was observed in group A , B and C as compared with the control group and are statistically significant (p=0.000 ; Cl=95% each). The same was observed in the 4th week although Group A (0.061852±0.00052; p=0.00) recorded lowest bilirubin level while group C (0.06707±0 .00040; p=0.00) has the highest bilirubin level as compared with control group (table 17).

3.8. Effects of administration of STBH on Histological parameters

Table 1 Effects of administration of STBH on the Weight Of the Rats

Groups		Duration in Weeks/Weight of rats (g)						
		Acclimatisation	2	3	4	5	WG	PWG(%)
		(n=12)	(n=12)	(n=9)	(n=6)	(n=3)		
A	75.67±7.05	100.88±8.48	103.73±3.61	119.14±7.93	132.10±11.51	192.83±23.25	64.75	50.55
B	74.15±6.75	93.38±8.94	98.79±11.17	112.59±15.07	131.35±25.08	186.00±55.83	56.67	43.81
C	74.62±9.33	98.98±17.95	97.34±10.79	102.71±7.37	121.20±2.82	165.50±2.59	45.38	37.77
D	85.62±5.42	113.50±3.32	118.14±3.47	135.23±4.23	158.90±1.27	184.00±0.00	49.38	36.68

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated, WG= Weight gain, PWG= %weight gain; Values are mean ± standard deviation. (Mean value along the column with same letter are not statistical differ from each other at p<0.05)

Table 2 Effects of administration of STBH on Pack cell Volume (PCV) of the Rats

Group	PCV(week 1)	PCV (week 2)	PCV(week 3)	PCV(week 4)
A	37.00±2.00^{a}	46.00±2.00^{c}	46.00±1.00^{c}	47.67±0.58^{c}
B	40.00±1.00^{b}	41.33±1.53^{b}	41.67±1.53^{b}	43.00±1.00^{b}
C	39.00±1.00^{ab}	37.00±2.00^{a}	38.00±1.00^{a}	39.33±0,58^{a}
D	37.00±0,00^{a}	44.00±0.00^{bc}	36.00±0.00^{a}	38.00±0.00^{a}

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated, PCV= Paked cell volume. Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05.

Histological study of the heart, liver and the kidney of the rats showed a near normal architecture. Although hyalinization (H), increased urinary pore (U) and degeneration of renal tubules were observed in Figure 2 of the kidney,

also the heart slide (Figure 3) showed hyalinization of cardiac cells (arrow) and damaged muscle fibers. The defects observed in this present study were not generalized indicating that the defects were not caused by STBH.

Table 3 Effects of administration of STBH on Red Blood Cell (RBC) of the Rats

Group	RBC 10^6/uL (Week 1)	RBC 10^6/uL (Week 2)	RBC 10^6/uL (Week 3)	RBC 10^6/uL (Week 4)
A	5.92±0.02^a	7.12±0.29^b	7.16±0.08^c	7.09±0.05^d
B	6.50±0.20^b	6.53±0.04^a	6.51±0.35^c	6.51±0.03^c
C	6.50±0.10^b	6.37±0.12^a	6.37±0.12^b	6.24±0.144^b
D	6.6.±0.00^b	7.12±0.00^b	5.72±0.00^a	5.68±0.00^a

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated.
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at $p<0.05$.

Table 4 Effects of administration of STBH on Hemoglobin (HGB) of the rates

Group	Haemoglobin g/dL (Week1)	Haemoglobin g/dL (Week2)	Haemoglobin g/dL (Week3)	Haemoglobin g/dL (Week4)
A	12.33±0.67^a	15.33±.67^c	15.33±0.34^c	15.89±0.19^c
B	13.33±0.34^b	13.78±0.51^b	13.89±0.51^b	14.33±0.34^b
C	13.00±0.00^{ab}	12.33±0.67^{bc}	12.67±0.34^b	13.11±1.9^a
D	12.33±0.00^a	14.67±0.00^c	12.00±0.00^a	12.67±0.00^a

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated, WG= Weight gain, PWG= %weight gain. Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at $p<0.05$.

Table 5 Effects of administration of STBH on Mean Cell Volume (MCV) of the rats

Group	MCV fl (Week 1)	MCV fl (Week 2)	MCV fl (Week 3)	MCV fl (Week 4)
A	62.50±3.48^b	64.60±0.00^b	64.25±1.85^b	67.26±0.35^b
B	61.56±1.77^b	63.32±1.95^b	64.03±2.01^b	66.01±1.35^b
C	60.00±1.35^{ab}	58.09±3.18^a	59.65±1.01^a	63.01±0.54^a
D	56.06±0.00^a	61.8±0.00^a	62.94±0.00^b	66.90±0.00^b

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated.
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at $p<0.05$.

Table 6 Effects of administration of STBH on Mean Cell Hemoglobin of the rate

Group	MCH pg/cell (Week 1)	MCH pg cell(Week 2)	MCH pgcell (Week 3)	MCH pgcell (Week 4)
A	20.83±1.16^b	21.54±0.10^b	21.42±0.62^b	22.42±0.11^b
B	20.52±0.59^b	21.11±0.65^b	21.35±0.67^b	22.00±0.45^b
C	20.00±0.45^{ab}	19.37±1.06^a	19.88±0.34^a	21.00±0.17^a
D	18.69±0.00^a	20.60±0.00^a	20.98±0.00^b	22.30±0.00^b

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated.
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at $p<0.05$.

Table 7 Effects of administration of STBH on Mean Cell Hemoglobin Concentration (MCHC) of the rats

Groups	MCHC g/dL (week 1)	MCHC g/dL (week 2)	MCHC g/dL (week 3)	MCHC g/dL (week 4)
A	33.326±0.01[a]	33.327±0.01[a]	33.327±0.01[a]	33.326±0.01[a]
B	33.323±0.01[a]	33.323±0.01[a]	33.327±0.01[a]	33.323±0.01[a]
C	33.325±0.01[a]	33.327±0.06[a]	33.33±0.00[a]	33.327±0.01[a]
D	33.325±0.01[a]	33.325±0.01[a]	33.32±0.014[a]	3.320±0.014[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated.
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05.

Table 8 Effects of administration of STBH on White Blood Cell (WBC) of the rats

Group	WBC x10^9/L	WBC x109/L	WBC x10^9/L	WBC x109L
A	11.30±0.20[a]	16.77±0.25[b]	6.44±0.07[b]	7.167±0.06[b]
B	11.93±0.15[b]	18.27±0.57[c]	8.467±0.31[d]	8.30±0.10[c]
C	18.27±0.40[c]	11.80±0.36[a]	7.98±0.14[c]	9.07±0.06[d]
D	12.20±0.00[b]	11.50±0.00[a]	5.31±0.00[a]	5.40±0.00[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated.
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05.

Table 9 Effects of administration of STBH on Lymphocytes level of the rats

Group	Lymphocytes10^9/L (Week 1)	Lymphocytes10^9/L (Week 2)	Lymphocytes10^9/L (Week 3)	Lymphocytes10^9/L (Week 4)
A	8.11±0.11[a]	11.23±2.62[ab]	6.22±1.73[b]	6.46±0.67[b]
B	8.39±0.07[b]	13.88±0.51[b]	7.06±0.25[b]	7.25±0.05[c]
C	14.43±0.14[d]	9.78±0.21[a]	7.86±1.85[b]	7.98±0.14[c]
D	9.15±0.00[c]	8.63±0.00[a]	3.24±0.00[a]	3.56±0.00[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated. Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05)

Table 10 Effects of administration of STBH on Monocytes level of the Rats

Group	Monocytes 109/L (Week 1)	Monocytes 109/L (Week 2)	Monocytes 109/L (Week 3)	Monocytes 109/L (Week 4)
A	0.19±0.07[a]	0.23±0.98[a]	0.87±0.4[a]	0.11±0.03[a]
B	0.16±0.07[a]	0.25±0.12[a]	0.11±0.44[a]	0.11±0.05[a]
C	0.36±0.00[b]	0.19±0.67[a]	0.08±0.00[a]	0.12±0.05[a]
D	0.12±0.00[a]	0.12±0.00[a]	0.11±0.00[a]	0.11±0.00[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05)

Table 11 Effects of administration of STBH on Granulocytes Percentage of the rats

Group	Granulocytes 10^9//L (Week 1)	Granulocytes 10^9/L (Week 2)	Granulocytes 10^9//L (Week 3)	Granulocytes 10^9//L (Week 4)
A	2.79±0.12[a]	3.91±0.52[b]	1.59±0.92[a]	0.87±0.106[a]
B	3.26±0.11[b]	3.95±0.15[c]	1.22±0.09[a]	0.86±0.18[a]
C	3.35±0.27[b]	1.85±0.18[a]	0.96±0.09[a]	0.88±0.13[a]
D	2.81±0.00[a]	2.65±0.00[b]	1.91±0.55[a]	1.67±0.00[b]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05

Table 12 Effects of administration of STBH on Eosinophil of the rat

Group	Eosinophil10^3/L (Week 1)	Eosinophil10^3/L (Week 2)	Eosinophil10^3/L (Week 3)	Eosinophil10^3/L (Week 4)
A	0.11±0.00[a]	0.17±0.00[b]	0.06±0.00[b]	0.07±0.00b
B	0.12±0.00[b]	0.18±0.01[c]	0 .08±0.00[d]	0.40±0.00[c]
C	0.18±0.00[c]	0.12±0.00[a]	0.79±0.00[c]	0.09±0.00[d]
D	0.12±0.00[b]	1.00±0.00[b]	0.05±0.00[a]	0.05±0.00[d]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05

Table 13 Effects of administration of STBH on Alanine Aminotransferase (ALT) level of the Rats

Group	ALT U/L (Week 1)	ALT U/L (Week 2)	ALT U/L (Week 3)	ALT U/L (Week 4)
A	19.43±0.83[a]	21.87±0.31[a]	22.700±0.66[a]	23.17±1.53[a]
B	23.90±1.03[b]	23.97±0.72[b]	26.63±0.41[c]	27.17±0.32[b]
C	26.07±0.75[c]	31.10±0.75[c]	34.13±0.35[d]	35.27±0.68[c]
D	20.60±0.00[a]	23.80±0.00[b]	25.50±0.00[b]	25.80±0.00[b]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05

Table 14 Effects of administration of STBH on Aspartate Aminotransferase (AST) level of the Rats

Group	AST U/L (Week1)	AST U/L (Week2)	AST U/L (Week3)	AST U/L (Week4)
A	63.20±20.96[a]	78.00±3.01[a]	78.20±0.26[b]	80.80±0.46[b]
B	77.06±0.96[a]	78.63±2.59[a]	80.03±0.67[c]	83.40±0.75[c]
C	79.40±0.9[a]	77.87±2.69[a]	84.17±0.84[d]	85.87±0.31[d]
D	74.60±0.28[a]	77.23±0.07[a]	75.60±0.28[a]	75.70±0.14[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05

Table 15 Effects of administration of STBH on Creatinine (CREA) level of the Rats

Group	CREA mg/dl (Week 1)	CREA mg/dl (Week 2)	CREA mg/dl (Week 3)	CREA mg/dl (Week 4)
A	0.46±0.45[a]	0.22±0.11[a]	0.24±0.00[b]	0.28±0.00[b]
B	0.24±0.01[a]	0.27±0.01[b]	0.31±0.00[c]	0.37±0.01[c]
C	0.27±0.00[a]	0.31±0.02[c]	0.380±0.01[d]	0.41±0.02[d]
D	0.22±0.03[a]	0.22±0.01[a]	0.21±0.01[a]	0.22±0.01[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05

Table 16 Effects of administration of STBH on Urea Level of the Rats

Group	UREA mg/dl	UREA mg/dl	UREA mg/dl	UREA mg/dl
A	13.3903± 0.32088[a]	13.73685± 0.20643[a]	14.53342±0.51694[a]	15.11033±0.07851[a]
B	15.05113± 0.27928[b]	15.52219± 0.10264[b]	16.21520±0.11077[c]	18.63477±0.10038[b]
C	16.84885± 0.23326[c]	17.11920±0.36568[c]	18.17346±0.06387[d]	21.04943±0.43232[c]
D	15.02955± 1.41421[b]	15.16164±1.41421[b]	15.32509±1.41421[b]	15.21745±7.07106[a]

Values are mean ± standard deviation. . (Mean value across the Colum with same letter are not statistical differ from each other at p<0.05)

Table 17 Effects of administration of STBH on Bilirubin Level of the Rats

Group	BILIRN mg/dl	BILIRN mg/dl	BILIRN mg/dl	BILIRN mg/dl
A	0.05237±0.00038[a]	0.05724±0.0007494[a]	0.06179± 0.00065[b]	0.061852±0.00052[b]
B	0.05413±0. 00153[b]	0.05637±0.00274[a]	0.06342±0. 00036[c]	0.06375±0.00058[c]
C	0.05527±0. 000079[b]	0.06247±0 .00119[b]	0.06529±0.00023[d]	0.06707±0 .00040[d]
D	0.05533±0.000001[b]	0.05673±0.000001[a]	0.05610±0.000001[a]	0.06±0.000001[a]

A=Group treated with 0.65mg STHB, B= Group treated with 1.3mg STHB, C= Group treated with 2.6mg of STHB, D= Untreated
Values are mean ± standard deviation. Mean value along the column with same letter are not statistical differ from each other at p<0.05)

3.9. Effects of administration of STBH on Liver, Kidney and Heart

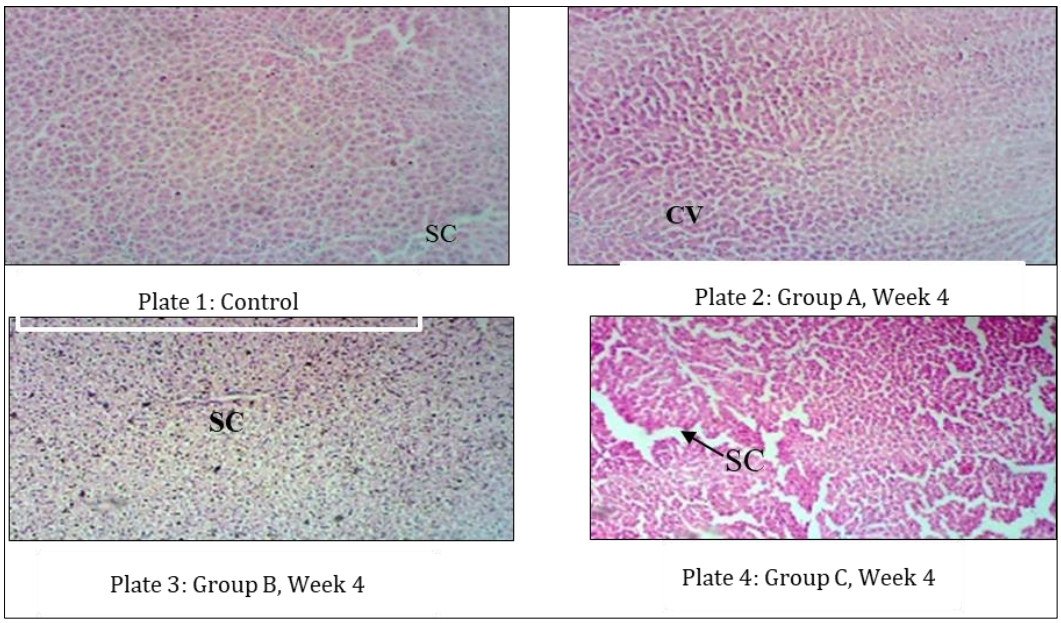

Figure 1 Photomicrograph of the liver of rats after 4 weeks feeding (×100 (H&E stains). Central vein (CV). Sinusoids capillary (SC),

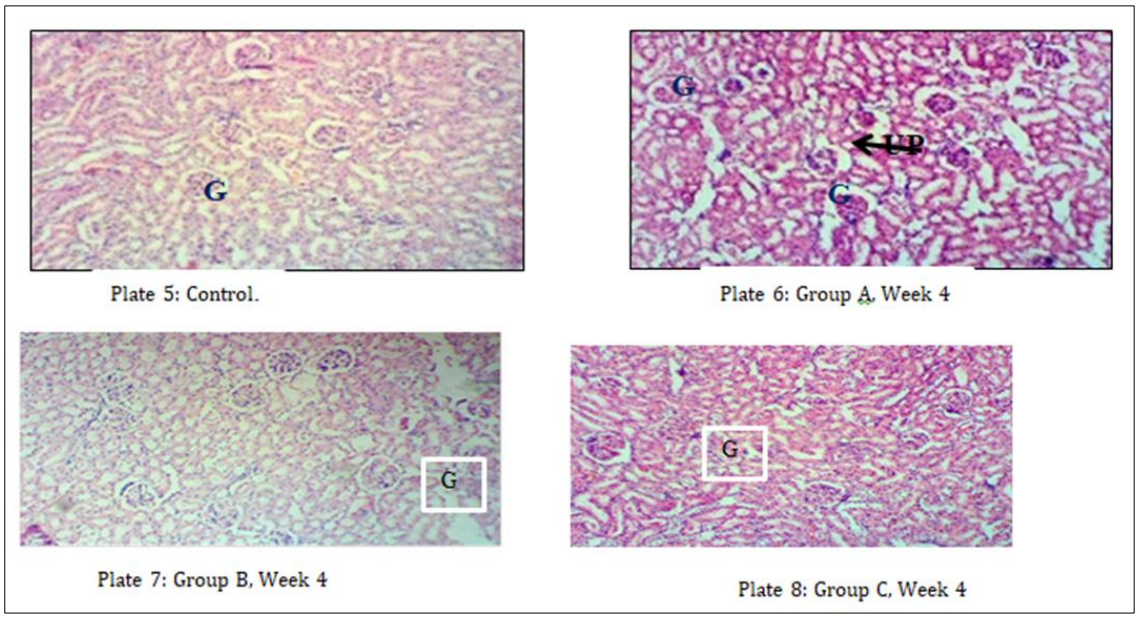

Figure 2 Photomicrograph of the Kidney of after 4 week of feeding (×100 (H&E stains). Kidney sections with almost near architecture. Urinary pore (U), glomerular (G), renal tubules are all intact

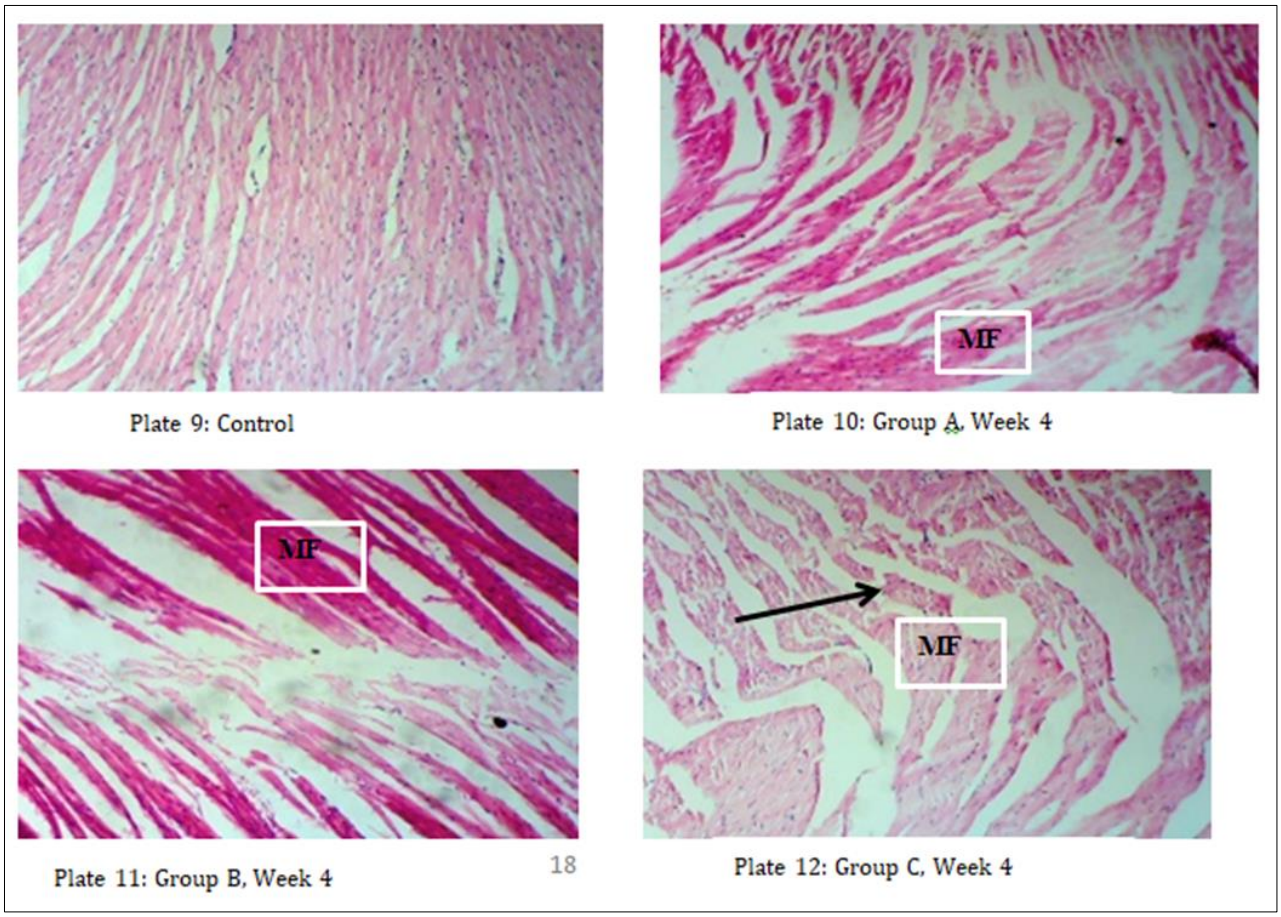

Figure 3 Photomicrograph of the Heart of rats treated with STBH after 4 week (×100 (H&E stains). Heart sections with almost near architecture but with showing hyalinization of cardiac cells. Muscle fibre (MF),

4. Discussion

Sorghum bicolor L.*, Curcuma longa* and *Bridelia ferruginea* are plants that are being used in folklore medicine in the management of various ailments. So also is honey especially in respiratory related complaints and wound treatment. However, this study is the first to assess the effects of the mixture of all the plants used in this investigation in honey on the haematological indices, biochemical profile and histological profile of apparently healthy rats.

A total of 40 male rats were used for the study. There was progressive and statistically significant ($p<0.05$) weight gain across the groups. Comparing the percentage of weight gain of the groups at the end of 28 days of administration of the herbal mixture, Group A (50.55%) has the highest percentage weight gain followed by group B (43.81%), group C (37.77%), and the least in control group (36.68%). This shows that the herbal mixture has effect on the weight of the experimental group as compared to the control group. The presence of turmeric and honey in feeds has been reported by several researchers to have positive effect on the body weight [32, 33]. Moreover, the addition of *Curcuma longa* to many diet have also been reported to enhance the digestion rate and absorption of many nutrients in the diets that might result in greater efficiency in the utilization of feed, resulting in enhanced growth [34]. The present study shows that the administration of the herbal mixture at low dosage (0.65mg/g) has high improvement in the body weight of the rats compared to the control. Iwueke *et al.* [35] stated that intake of *Curcuma longa* at different concentrations may improve metabolic status through balancing of basal metabolic rate, which may in turn cause increased energy intake. The weight gain in experimental animals in this study therefore is attributed to high nutritional value of the herbal mixture, digestibility and acceptability of the herbal mixture by animals as well as presence of non- toxic compounds in the food [35].

Haematological assay was conducted to investigate the possible effects of the herbal mixture on the morphology of the cell components of the blood and physiological status. The investigation helps to identify improvement or any harm caused by the administration of the herbal mixture. According to Seibel *et al.* [36] change in blood constituents has it effect on the physiological wellbeing and are diagnostically important in routine clinical evaluation of the state of health. The PCV level of the rat group fed with different dosages (0.65mg/g, 1.3mg/g and 2.6mg/g) of STBH were significantly ($p<0.05$) higher compared to the control group. A similar result was reported by Rahmani *et al.* [16] who worked on turmeric and Ayuba *et al.* [19] and Sènou [37] who reported erythropoietin stimulating effect of S*orghum bicolor.* It should be noted that these levels are within the reference range (9.40 to 17.90 g/dL) according to Campbell [38]. Adequate PCV level in the body indicates the absence of normocytic anaemia. This is in correlation with the nutritional status of animals and adequate protein in the STBH [39].

The increase in the RBC of the rat fed with STBH in this present study could be attributed to the ability of the leaves of *Sorghum bicolor* to stimulate erythropoiesis in a specific way and in a dose dependent manner [37]. Toshiaki *et al*. [40] stated that the RBC in rats increase sharply with age until about 10 weeks and steadily till 18 weeks of age and then decline towards 58 week of age. Thus the present result of RBC is in order based on the age range of the rats.

The increase in Hemoglobin (HGB) among the rats fed with STBH as compared to the control shows that STBH did not interfere with the ability of RBC in carrying oxygen. This is in contrast with the report of Adesola *et al.* [12] who noted inhibitory effect of *Bridelia ferruginea* on iron (II) sulphate which is an important part of the blood but agree with Awodele *et al.* [41] who reported no decrease in HGB at 4000 mg/kg. The normalized level of hemoglobin recorded in this current study may indicate that the STBH contain phytochemicals and compounds that stimulate the secretion or formation of erythropoietin in the stem cells of normal rats. Contrary to decline in hemoglobin reported in other literature. Stimulation of stem cells in the bone marrow to produce red blood cells occurs due to the action of erythropoietin which is a glycoprotein hormone [42, 43].

The observed level of MCHC, MCH and MCV level derived in this study did not differ significantly from the report of Konmy and Doko-Allou *et al*. [44]. The value obtained in the MCHC and MCH of the rat fed with STBH showed that there is no deformation in the RBC. This is in line with the report of Oduola *et al.* [45] and Konmy *et al*. [44]. The mean value of MCV in this current study was within normal rang thus indicated the absence of microcytic anaemia, a condition in which the red blood cells (erythrocytes) contain less hemoglobin and are usually also hypochromic, meaning that the red blood cells appear paler than usual [46]. This is in contrast to the reported of Iwueke *et al.* [35] who observed slight reduction in MCV level of rat treated with turmeric powder. The correlation in the increase pattern of PCV and MCV in this study indicate production of healthy RBC [47, 48]

The increase level of WBC, Lymphocytes and granulocytes in rats fed with STBH although not above the basal, reflect its immunostimulatory potential. Researchers have reported individual effects of *Sorghum bicolor* L*.,Curcuma longa* , *Bridelia ferruginea* or honey in experimental rats resulting in increased total white blood cell count [41, 49]. This is in

agreement with Muriithi *et al.* [42]. This indicates that STBH has capacity of stimulating the animals' defensive mechanism. According to Adeosun *et al*. [50], high level of WBC enhances a strong resistance in the animal. The result of thids study agrees with Benson *et al.* [18] who observed anti-inflammatory and immune-modulating properties of *Sorghum bicolor* leaf in an *in vitro* study.

Liver biomarkers (AST and ALT) are used to evaluate liver cytolysis of which ALT has been a more sensitive biomarker of hepatotoxicity than AST [44]. In this present study, ALT and AST level were found to be within the reference range as described by David *et al.* [51]. Although the highest mean level was observed in group C(2.3mg/g), this was still lower compared to the report of Olayode *et al*. [48] and Delwatta *et al*. [52] who noticed a significantly high mean level of AST and ALT among apparently health individuals. The AST and ALT in this current investigation is supported by the near normal liver architecture of the histological result. Similar result was obtained by Sènou *et al*. [3]. Tumeric has been report to have potential to protect the liver against oxidation damage and diseases arising from drug damages (especially by acetaminophen and cancer drugs), infection such as hepatitis A, B, C, E, alcohol damage, fatty liver, cirrhosis and cancer has being reported by Singh *et al.* [15].

There is significant ($p<0.05$) reduction in creatinine level while urea level was slightly higher among the rats fed with the STBH. The Urea level in this present study was however higher than the one reported by Sènou *et al.* [3]. A reverse case was however noticed by Awodele *et al.,* [41] who observed reduction in urea level and no significant reduction in creatinine level with no histological defect.

The insignificant cytotoxicity observed in various organs could be partly due the antioxidant protection of bioactive compound present in STBH. Previous reports have shown *Sorghum bicolor* L*.,Curcuma longa* , *Bridelia ferruginea* and honey are good sources of antioxidants [37,53, 54].

5. Conclusion

The present study has revealed the safety of the formulated herbal mixture made from *Sorghum bicolor* L*. Curcuma longa*, *Bridelia ferruginea* and honey (STBH) administrated at different dosages. STBH significantly ($p< 0.05$), improves the haematological indices with no significant alteration of the liver, kidney and the heart architecture. Although, no obvious defects or death was noticed among the group fed with difference dosages of STBH worked on throughout the experimentation, it is however recommended that a low dosage of STBH (0.65mg/g - 1.3mg/g. of body weight) should be employed. The lipid peroxidation effect reported in *Bridelia ferruginea* [41] was eliminated through the synergetic effect of the component of STBH. Moreover, the ability of STBH to stimulate production of RBC and increase hemoglobin is an indication of its potential to treat anaemia. Furthermore, since STBH also has the ability to improve the level of PCV it could be exploited as a good therapeutic agent for management and prevention of aplastic anaemia and some thalassemia syndromes.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors report no conflicts of interest in this work.

Statement of ethical approval

Ethical approval for this research was obtained from Research Ethical Committee of Ekiti State University Teaching Hospital where the clinical aspect was conducted.

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