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Toxicological and Positive Changes in Hematological Pattern of Albino Rats Treated with Termitomyces Titanicus: Potential Valorization of an Indigenous Practice in the Treatment of Oris Cancrum (Noma) in Nigeria

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ABSTRACT

A neglected disease affecting many malnourished children and immunosuppressed patients in sub Saharan Africa is noma caused mainly by Fusobacterium necrophorum. Poverty and malnutrition are highly implicated in the epidemiology, prevalence and poor management of noma. Local mushroomsof the gens Termitomyces have been used in parts of northern Nigeria and Cameroon to improve health of noma patients, but detail studies to authenticate the effect of this medicinal mushroom on specific chronic diseases such as noma remain inadequate. Blood transfusion remains one of the critical palliative care strategies to manage anemia in noma disease. The current preliminary study was undertaken to establish the effect of a strain of termitomyces titanicus mushroom, which has been used in Nigerian and Cameroonian folk medicine in Itas Gadau Local government and parts of Bauchi State and North west Region respectively for the management of anemia, using hematological parameters such as hemoglobin level, white blood cell stabilization and packed cell volume of albino rats and also to ascertain its safety for consumption using liver enzyme profiling. The results indicated that rats treated with Termitomyces titanicus had a higher hemoglobin level (12.2 g/dl) compared to a treatment with vitamin B complex (11.3 g/dl) and untreated (10.1 g/dl). A significant increase in the total white blood cell level (26300cells/mm3) was observed with rats treated with mushrooms compared with 7500 cells/mm3 for rats treated with vitamin B complex. Hematological parameters showed a significant difference amongst the test group compared with the controls group. The results further revealed that rats treated with termitomyces had a cleaner liver than with rats treated with vitamin B complex. The results authenticate the practice of using this mushroom in the local management of anemic conditions in Oris cancrum (noma) patients and other chronic disease conditions such as cancer.

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Introduction

Chronic diseases such as oris cancrum (noma) and cancer generates a critical anemia which requires periodic dosing with hemotonics and in some cases blood transfusions. In tropical conditions, managing anemia in such disease situations is clinically challenging, with poor blood banking systems plagued by insufficient blood donors and poor storage conditions (Leslie, 2013). Anemia and low haemoglobil levels in noma disease condition is a widespread especially amongst the elderly, children and amongst cancer patients (Stoltzfus, 2003). WHO estimates the number people worldwide suffering from of anemia to be a staggering 3.5 billion in developing countries and that approximately 50% of all anemia cases can be attributed to iron deficiency [WHO/UNICEF, 2004], while epidemiological data on noma are scarce, but a current estimate of the global incidence is 30,000–40,000 cases per year, with a mortality rate of approximately 85% and a burden of disease calculated to be a loss of 1–10 million disability-adjusted life years. The global distribution of the disease burden of Iron deficiency anemia is heavily concentrated in Africa and WHO Regional Southeast Asia (Leslie, 2013). These regions bear 71% of the global mortality burden and 65 % of the disability-adjusted life years lost (Stoltzfus, 2003).The most highly affected population groups in developing countries are pregnant women (56%), school age children (53%), non-pregnant women (44%), and preschool children (42%). This exacerbated in patients with noma.

Figure 1: A patient with Oris Cancrum (Noma) Disease

Noma does not start as a necrotizing process, it is preceded by a small intraoral ulcer, an aphtous lesion or, frequently by acute necrotizing gingivitis (ANG). ANG is characterized by spontaneous bleeding, ulceration of the gingival papillae, pain and sometimes, greyish pseudomembranes for chronic cases, the blood picture is indicative of hematological changes such as white blood cells are not fully developed called blast cells. Symptoms may include bleeding and bruising problems, feeling tired, fever, and an increased risk of infections. These symptoms occur due to a lack of normal blood cells, with diagnosis typically made by blood tests or bone marrow biopsy. The exact cause of leukemia is unknown [17].

Although there are various drugs used for the treatment of anemia such as vitamin b complex, ferrous sulphate, in the clinical management of anemia in noma in Africa, they are not affordable to many poor people, and above all, the response with patients is also generally slow, and this trend is similar in most developing countries. In addition, the rural populations in various parts of the world do not have adequate access to high quality drugs for the treatment of anemia and noma, so these populations depend heavily on plants and herbal products for the treatment of noma and the attending anemia [17].

Mushrooms have served as a source of nutrition, medicine, and complete the ecosystem with recycling of nutrients. Mushrooms have been reported to be widely used in many local diets of the people of Katagum, Alkaleri, Dambam, Giade, Ganjuwa, Darazo, Dass, Ningi, Shira, Gamawa, Itas/Gadau of Bauchi State and a number of tribes in northern and north western Cameroon recipes [23]. Termitomyces titanicus and T robusta are widespread in Bauchi State and in northern and western Cameroon (Pegler &Pearce) one of the largest mushroom cap in the world is found in the West Africa region. It has a unique symbiotic relationship with termites [36]. The termites carry the fungal material down to their nests. There, plant material that cannot be digested by the termites is broken down by the fungus for the termites to eat. It is basidiomycetes in the family of Lyophyllaceae and grows up to three feet in diameter [37]. Novel cerebroside and termitomycespin1 have been identified in Termitomyces titanicus mushrooms [14]. Bioactive compounds from the genus termitomyces have shown anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents [13,14,15]. According to Bhanja et al, a number of immune-stimulating B-glucans have been isolated from Termitomyces robustus var titanicus [11]. The PS -1 beta glucans are water soluble while the PS-2 beta glucans are water insoluble, as well as repeating chains of polysaccharide and all isolated from the fruiting bodies of Termitomyces with the potential to activate macrophages.

In Nigeria, this mushroom is often ground and a paste made of it and given to underweight children and also children with diagnosed with anemia and children suffering with noma. In this paper, the results of the evaluation of the effect of Termitomyces titanicus mushroom on the hematological indices of albino rats with implication in the potential management of anemia and oris cancrum (noma) are presented.

Materials and Methods

Research Design

The experiment was designed in such a way that fresh Termitomyces titanicus mushrooms were collected from Itas Gadau, Bauchi State, Nigeria, Baligham, Ndop plains in the North West region of Cameroon, and authenticated by Botanists at the centre for Agronomic Research (IRAD) Bambili and aided by methods described earlier by Tibuhwa, rinsed in clean water, dried and ground to powder form [36]. The powder was then kept in brown khaki envelopes, away from sunlight.

Figure 2a: Termitomyces Titanicus (From the North West Region, Cameroon)

Figure 2b: Termitomyces titanicus from Itas /Gadau, Bauchi State, Nigeria

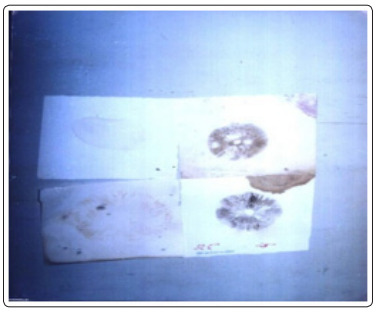


Figure 3: Spore print (from Fig1b above)

The different test groups of Laboratory animals were fed with the samples of Termitomyces titanicus, positive control lab animals treated on with vitamin B complex and negative controls being albino rats treated with normal feed with no test samples. Normal feed being made of corn and soy beans. This run for 8 days and each bled for blood samples. Samples were then analyzed for hematological values manually and with a hemo-analysing machine. Gross anatomy was done to verify for signs of organ toxicity. The entire study was done at the Phytobiotechnology Research Foundation laboratory Institute, Bamenda, Cameroon, from June to July 2016.

Methods

A dose of 25 g/day/rat of Termitomyces titanicus dried powdered leaf was considered to be the minimum dosage to be given per day. A total of 6 rats (of approximately the same age: 3.5 to 4 months and weight (300 g) grouped into 3 groups were identified as test, positive control and negative control (i.e2 rats per group). To the negative control, only the feed (25 mg/group/day) was administered for the duration of feeding. The positive control mice received a mixture of 1 tablet of Vitamin B complex (200 mg of ferrous sulphate and 0.25 mg of folic acid) and 25 g of normal feed/day. To the test group a 1:2 preparation of 65 g of powdered plant and 130 g of normal feed was made from which 25 g was administered orally/day, moistened with clean water. Administration was promptly done every morning.

Figure 3: (Test rats treated with Vit B complex)

After six days administration, blood samples were obtained by exsanguinations and put into lithium heparin test tubes for hematological examination. All rats were examined carefully for gross pathologic changes, with particular emphasis on the liver, kidney, lungs, heart and spleen [22].

Effects on Hematological parameters

All values were done using automatic analyzers and results confirmed by manual methods. The mean values for each parameter were established. All tests were done in duplicate to minimize error.

Effect on Packed cell volume (PCV)

The Packed Cell Volume, also called hematocrit, was used to calculate the Mean Cell Haemoglobin Concentration (MCHC) and Mean Cell Volume (MCV). These red cell indices are useful in the differentiation for the different types of anemia especially in leukemic condition.

The Packed Cell Volume is the proportion of total blood occupied by the red cells, expressed as a ratio. Anticoagulated blood in a glass capillary of specified length bore size and wall thickness is centrifuged in microhematocrit centrifuge at 12000-15000rpm for 3-5 minutes to obtain constant packing of the red cells. A small amount of plasma remains trapped between the packed red cells. The PCV value is read from the scale of a microhematocrit reader or calculated by dividing the height of the red cells column by the total column of blood.

Aplain capillary was filled with well-mixed anticoagulated blood to about three quarters. Using a sealant material, the indicated end of the capillary tube was sealed. The filled capillary in one of the numbered slots of the microhematocrit rotor was carefully located with the sealed end against the rim gasket (to prevent breakage). Centrifuge for 3-5 minutes (12000-15000rpm), using the shorter time when the rpm is 15000g. Immediately after centrifuging, the PCV was read off. Caution was exercised by first checking that there was no leakage of blood from the capillary. To read the PCV in a hand-held microhematocrit reader, the base of the cell column (above the sealant) was aligned on the "O" line and the top of the plasma on line 100. The PCV read off from the scale. The reading point is the top of the red cell column, just below the buffy coat layer (WBC and platelets).

Effect on Hemoglobin (Hb)

Hemoglobin is the oxygen-carrying element in the body. They are heterogeneous proteins produced in developing erythroblasts. Each human Hemoglob in (Hb) consists of a tetramer of 2α and 2β globin chains bound to a single heme moiety. Heme contains one ferrous iron (Fe2+) atom carried in a porphyrin ring.From the relation PCV= 3×Hb, the Hb was directly calculated for each sample indicating that Hb=PCV/3.

$$\text{Hb}\,(\text{g/dl}) = \frac{\text{PCV}\,(\%)}{3}$$

Effect on White Blood Cell (WBC)

In this case, the WBC count was used to investigate possible leucopenia or leukocytosis due to the presence of the feed, and not merely the investigation of infections. Whole blood was diluted 1 in 20 in an acid reagent which haemolyzes the red cells (not the nucleus of nucleated red cells), leaving the white cells to be counted. White cells are counted microscopically using an improved neubauer ruled counting chamber (haemocytometer) and the number of WBCs per liter of blood calculated.

A 0.35ml of the diluting fluid into a term tube using the 1ml graduated pipette was prepared. Using the 20ul pipette; 0.02ml of a sample of capillary blood or EDTA Venus blood was added. The blood was expelled three times in the diluting fluid by squeezing and releasing the rubber tubing. It was mixed by gently tapping the bottom of the tube a few times until the dilution of the blood now was 1:20. The test tube was gently tapped to mix the diluted blood. The counting chamber was filled with the diluted blood using Pasteur pipette. Air bubbles were avoided by not over flooding the counting chamber. The counting chamber was placed on the microscope stage and allowed 2 minutes for the cells to settle. The counting Chamber was then well focused. The cells in 4 large squares of the counting chamber were counted. The number of cells in 1ul f whole blood as follows was calculated as per the following equation:

$$WBC/\mu l = \frac{no\ of\ cells\ counted\ \times DF}{Area\ \times Depth\ of\ chamber}$$

Effect on PLATELETS Count

The blood was diluted with a 1% ammonium oxalate solution which completely haemolysis the red cells living only the platelets to be counted in the counting chamber.

A 0.38ml of diluting fluid was pipette into a test tube, filled in the 20ul pipette to the mark with blood. The content was expelled into the diluting fluid and washed out by drawing up the fluid and expelling it into the fluid a couple of times. The counting chamber was set up with its cover glass in position. Using the Pasteur pipette, the counting chamber was filled with one large drop of the diluted blood and then placed on the microscope stage for viewing using the 10x objective. The platelets in 4 corner squares

plus one middle square in the central roll area of the chamber were considered as per the following calculation:

Calculation

$$\text{Platelets}/\mu l = \frac{\text{No of platelets counted} \times DF}{\text{Area} \times \text{Depth of chamber}}$$

Effect on Differential blood count

The thin blood films were prepared, dried and fixed with 95 per cent methanol, and stained by giemsa method. Thereafter, the slides were dried and viewed under the microscope using the oil immersion objective.

Effect on Mean Cell Volume (MCV)

The mean cell haemoglobin from the blood samples of the rats was also determined. Mean cell haemoglobin (MCH) from albino rats treated with Termitomyces, positive and negative controls. This refers to the amount of haemoglobin in picograms (pg) in an average red cell was described for both treatment and controls. It was taken that when red cells are hypochromic, the MCH is reduced and when the cells are macrocytic, the MCH is increased.

While the Mean cell hemoglobin concentration (MCHC) was also determined and this refers to the concentration of haemoglobin in g/l in 1 litre of packed red cells was evaluated for all the treatments. When red cells are hypochromic and microcytic, the MCHC is reduced and usually calculated using the formulae below:

$$\text{MCV (Fl)} = \frac{\text{PCV (\%)} * 10}{\text{RBC (in millions)}}$$

$$\text{MCH (Pg)} = \frac{\text{Hb } \left(\frac{g}{dl}\right) * 10}{\text{RBC (in millions)}}$$

$$\text{MCHC (\%)} = \frac{\text{Hb } \left(\frac{g}{dl}\right) * 100}{\text{PCV (\%)}}$$

Preliminary toxicity studies on albino mice were carried out using 6 mice in each treatment in a total of three treatments for a three week period. Morphological aberrations in liver, kidney, heart or lungs was observed after dissection of the rats with these organs removed, displayed and examined using a hand lens. The weights of each of these organs had no significant difference from the control (untreated mice). No effect was observed on the red blood and white blood cells of the mice. The liver enzymes used as markers for liver anomaly, serum glutamate transminase (mean value of 40U/L) and serum oxalate transaminase (Mean value of 20 U/L) were slightly higher when compared to the control mice (mean value of 30U/L for SGOT and 15U/L for SGPT).The liver enzymes values for the mice treated with MP are within the normal range for a healthy human liver of less than 40U/L for both SGOT and SGPT).

Results

After 6 days of administration of Termitomyces titanicus sample, gross anatomical examination of the rats did not show any sign of toxicity. Some hematological parameters were not significantly different amongst tests and control groups (Figs 4, 5, 6 and 7) except for values of Hb, PCV and RBC count which were significantly different amongst the test and control groups. (Fig 2and Fig 3).

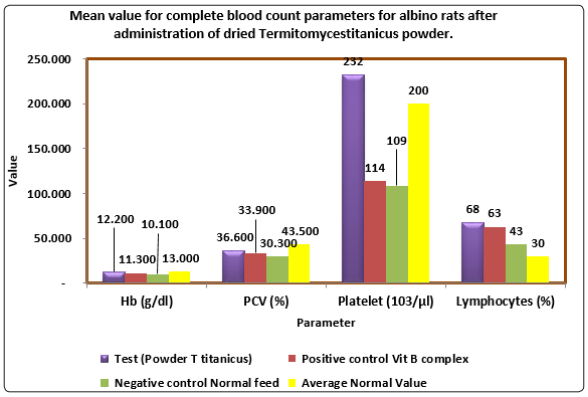


Figure 4: Mean value for complete blood count parameters for albino rats after administration of dried Termitomyces titanicus powder

The result showed that rats fed with Termitomyces titanicus generally showed increased haemoglobin levels, increased packed cell volume and increased lymphocytes counts.

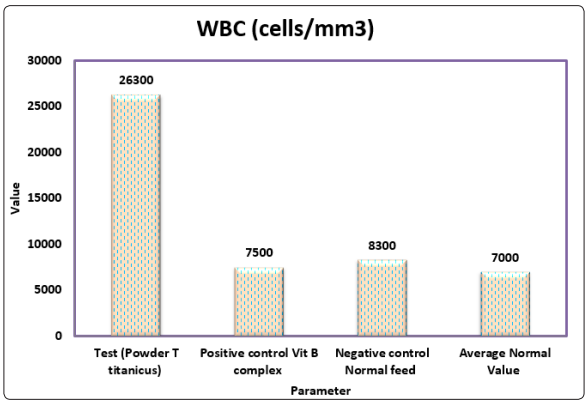


Figure 5: Mean value for changes in total white blood cell counts for albino rats after administration of dried Termitomyces titanicus powder

Increased white blood cell counts were observed in the test rats fed with Termitomyces titanicus when compared with the controls.

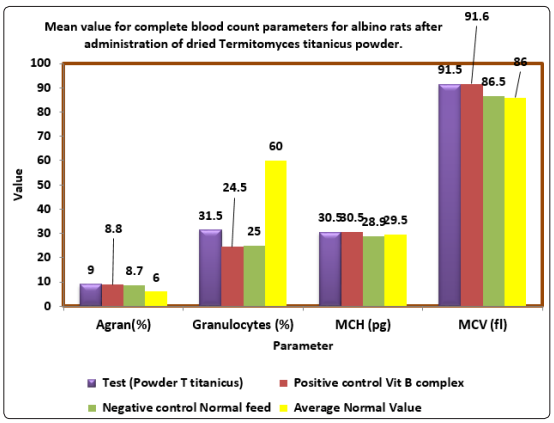


Figure 6: Mean value for complete blood count parameters for albino rats after administration of dried Termitomyces titanicus powder

Inferential statistical analysis using the one sample t test was used to test if there is a significant difference between mean value for complete blood count observed from the parameters and the normal value in humans (calculated as the average of the normal value in humans).

The result for each parameter is presented in the tables below

Hb (g/dl)

T Test: One Sample Hb (g/dl)

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	11.200	1.054	0.608	(2.959)	2.000	1.708	0.902

T TEST			Hyp Mean	13.000	
	p-value	t-crit	lower	upper	sig
One Tail	0.049	2.920			yes
Two Tail	0.098	4.303	8.583	13.817	no

PCV (%)

T Test: One Sample

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	33.600	3.161	1.825	(5.425)	2.000	3.132	0.968

T TEST			Hyp Mean	43.500	
	p-value	t-crit	lower	upper	sig
One Tail	0.016	2.920			yes
Two Tail	0.032	4.303	25.748	41.452	yes

Platelet (103/µl)

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	151.667	69.616	40.193	(1.203)	2.000	0.694	0.648

T TEST			Hyp Mean	200.000	
	p-value	t-crit	lower	upper	sig
One Tail	0.176	2.920			no
Two Tail	0.352	4.303	(21.268)	324.601	no

Lymphocytes (%)

T Test: One Sample

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	58.000	13.229	7.638	3.666	2.000	2.117	0.933

T TEST			Hyp Mean	30.000	
	p-value	t-crit	lower	upper	sig
One Tail	0.034	2.920			yes
Two Tail	0.067	4.303	25.138	90.862	no

Agran(%)

T Test: One Sample

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	8.833	0.153	0.088	32.127	2.000	18.549	0.999

T TEST			Hyp Mean	30.000	
	p-value	t-crit	lower	upper	sig
One Tail	0.000	2.920			yes
Two Tail	0.001	4.303	8.454	9.213	yes

Granulocytes (%)

T Test: One Sample

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	27.000	3.905	2.255	(14.637)	2.000	8.450	0.995

T TEST			Hyp Mean	30.000	
	p-value	t-crit	lower	upper	sig
One Tail	0.002	2.920			yes
Two Tail	0.005	4.303	17.299	36.701	yes

MCH (pg)

T Test: One Sample

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	29.967	0.924	0.533	0.875	2.000	0.505	0.526

T TEST			Hyp Mean	29.500	
	p-value	t-crit	lower	upper	sig
One Tail	0.237	2.920			no
Two Tail	0.474	4.303	27.672	32.261	no

MCV (fl)

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
3.000	89.867	2.916	1.684	2.000	2.000	1.155	0.816

T TEST			Hyp Mean	29.500	
	p-value	t-crit	lower	upper	sig
One Tail	0.092	2.920			no
Two Tail	0.184	4.303	82.623	97.111	no

WBC (cells/mm^3)

T Test: One Sample

SUMMARY			Alpha	0.050			
Count	Mean	Std Dev	Std Err	t	df	Cohen d	Effect r
			10,630.7				
3.000	14,033.333	73	6,137.680	1.146	2.000	0.662	0.630

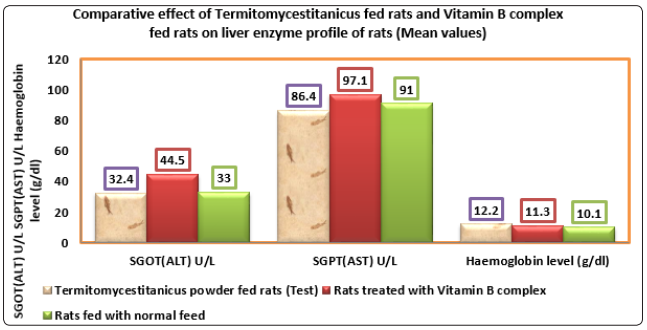
T TEST			Hyp Mean	29.500	
	p-value	t-crit	lower	upper	sig
One Tail	0.185	2.920			no
Two Tail	0.370	4.303	(12,374.971)	40,441.637	no

Figure 7 T test analysis result for significant difference between mean values for complete blood count observed from the parameters and the normal value in humans

The results of showed that the blood picture from blood drawn from the rats in all the treatment had no hemolysis. The blood picture for rats treated with Termitomyces titanicus showed marked normochromicity when compared to all rats from the positive and negative control groups. Liver function profile of treatment group with Vit B complex was higher than with test and negative control

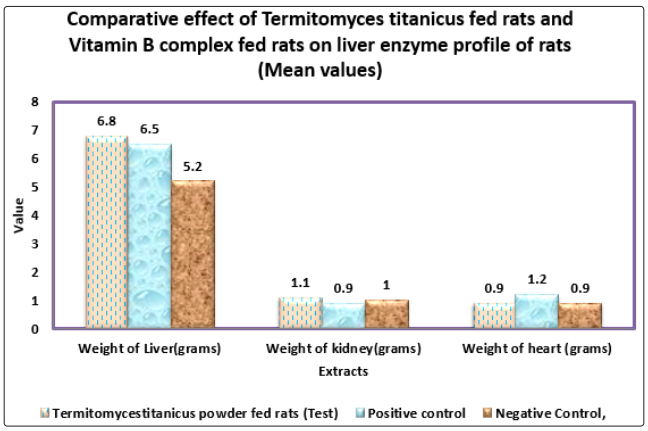
groups (Tables 1 and 2)

Table 1: Comparative effect of Termitomyces titanicus fed rats and Vitamin B complex fed rats on liver enzyme profile of rats (Mean values)



The results in Table 1 show that the weights of the organs from treatment with vitamin B complex were higher than with treatment from Termitomyces titanicus

Table 2: Comparative effect of Termitomyces titanicus fed rats and Vitamin B complex fed rats on the weight of the Liver, Kidney and heart (Mean values)



The results in Table 2 show that the comparative effect of Termitomyces titanicus fed rats and rats fed with vit b complex on liver enzyme profile of the test rats.

Discussion

The study has demonstrated that anemic condition in noma disease can be improved using the mushroom Termitomyces titanicus in tandem to the traditional practice of using the dried powder in pastries and food to improve patients with noma and other debilitating conditions in rural areas in Bauchi state in Nigeria and by the pastoralist in northern and north western Cameroon.

Noma (cancrum oris) is a devastating infectious disease that destroys the soft and hard tissues of the oral and para-oral structures; it remains the scourge of children and the "face of poverty" in Sub-Saharan Africa [3]. While the disease has been eliminated in Europe and North America (largely through control of malnutrition and infectious diseases), its incidence continues to increase in the underdeveloped countries of Sub-Saharan Africa. These countries (extending from Senegal, through Nigeria, to Ethiopia) account for most of the annual global cases of noma (estimated at 140,000) and are referred to as the "noma belt.

This preliminary study has shown the medical importance of Termitomyces titanicus mushroom as anti-anemic activity by improving the hemoglobin level (Hb) the red blood cell level (RBC), haematocrit level (HCT) and mean cell hemoglobin concentration (MCHC), values in the test rats as shown in Figures 4, 5, 6, 7, .significant elevation in the levels of Hb, RBC, HCT and MCHC agrees with previous reports that mushrooms are functional foods [13].

However, previous reports on mushrooms have highlighted more of the proximate nutrients such as crude protein, fibres and mineral elements in food. It is not always the case that when mushrooms are consumed the hemoglobin level is raised. This preliminary empirical evidence such as this which probes into how these mushroom nutrients change the blood picture has not been exploited in previous studies but not in the case management of oris cancrum-noma. These observations have not been previously observed for this mushroom but similar reports for other plants exist [12]. In this study, the mushroom fed rats had a Hemoglobin level of 12.2g/dl, higher than with vitamin B complex (11.3g/dl) which is frequently being prescribed by physicians in the treatment of anemic condition for patients with Oris cancrum.

From Figures 4, 5, 6 and 7, the WBC count for mushroom fed rate was 26,300 cells per mm3, while lower WBC counts were observed for rats administered with Vitamin B complex (7500cells per mm3). These findings suggest that Termitomyces titanicus may contain immune boosting ingredients. Immune boosting beta glucans and termitocesphin 1 have been reported from Termitomyces titanicus [11,14]. This probably corroborates the observations from local people in rural parts of Bauchi State in Nigeria and Cameroon who explained that the dried mushroom powder is made into a local pastry –pap and served as a nutrient support to patents with oro-facial deformities and with significant improvement in their health condition over time.

These compounds may have had an effect in increasing in white blood cells counts of rats fed on mushroom powder. Bhanja et al, reported that beta glucans and termitocesphin1 had an effect in activation of macrophages [11]. The observation in these studies probably lends credence to the traditional practice in Cameroon where dried powder of Termitomyces mushroom is given to underweight babies [16-35,38]. Increased platelet counts are probably due to the stress and trauma the mice went through during exsanguinations [1-10].

The giemsa stained blood smears for the test and control groups showed microcytic cells confirming the low MCV (Table 1) values for their samples even after manual calculations. The MCHC values for the two showed a normal range (Figures 4, 5, 6, and 7). The reason behind the reduction in the MCV value could be as a result of inadequate vitamin B12 supply in the test rats. The MCV and MCHC values in the negative control mice showed increase ranges (Figure 4) confirming the macrocytic cells in the giemsa stained smears. Gross anatomical examination of organs in situ after dissection revealed no significant change from the control mice indicative of the fact that Termitomyces titanicusis not toxic to the cells of the mice. The conclusion from this study is that Termitomyces titanicus reduces anemia in the test rats and is not toxic when consumed as observed in the test rats. This study was informed by a traditional practice in the management of oris cancrum where the powdered dried mushroom was applied to improve the health of sufferers. The findings have valorized this practice. More so, from the studies with the rats, the mushroom shows no toxic or lethal effect on the liver and kidneys compared to vitamin B complex control which is indicative that prolonged use of vitamin B complex may be unhealthy to the liver.

It is therefore recommended for further clinical studies to ascertain its use as nutritional support for patients with anemia and in the clinical management of oris cancrum (noma) in Nigeria and Cameroon.

Acknowledgement

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