

## Effects of Aqueous Leaf Extract of *Gongronema Latifolium* on the Histology of the Liver and Bone Marrow of Wistar Rat

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Received: 29/11/2016

Revised: 18/12/2016

Accepted: 30/12/2016

### ABSTRACT

*Gongronema latifolium* is a wide spread tropical rain forest plant in Africa, largely eaten locally as vegetable for food especially by those in the southern parts of Nigeria for its medicinal and nutritive value. The knowledge of its histologic effects particularly of the liver and bone marrow is not adequately documented. Fresh leaves of *Gongronema latifolium* were dried and ground to obtain 500g which was subjected to aqueous extraction and gave a yield of extract of 43g of fine powder of *G. latifolium*. Thirty (30) weaned wistar rat were used in the study. They were randomly divided into three (3) groups namely control, low dose and high dose respectively. The treatment groups were fed with the aqueous leaf extract alongside animal feed and clean water, while the control group was only fed with animal feed and clean water. The low dose and high dose groups were given 25% and 75% of the dosage consumed by users respectively for 21 days while control group was given water as placebo. The rats were sacrificed and the liver and bone marrow tissue harvested respectively. The liver tissue was fixed, processed, sectioned and stained using Haematoxylin and Eosin method. The bone marrow was aspirated from long bones, smears made, fixed and stained using Leishmann staining technique. The evidence of toxicity was observed more in the high dose group. The nuclei of the hepatocytes in this group showed mild enlargement and infiltration by polymorphonuclear leucocytes as well as increased production of white blood cells in the bone marrow suggesting an inflammatory effect on these organs.

**Keywords:** *Gongronema latifolium*, Liver, Bone marrow, Wistar rat, Histologic and Toxicity

### INTRODUCTION

The knowledge of the use of plants as food, condiments and medicine is as old as man. <sup>[1]</sup> Large populations of people, especially in the developing world, rely on folk medicines for the treatment of common infections as well as persistent diseases

*Gongronema latifolium* is a tropical rain forest plant which is widely spread in tropical Africa and from the family Asclepiadaceae. It is a slender climber often 3-4m long popularly referred to as *Utazi* in

Igbo, Arókéké in Yoruba and Utasi in Ibibio . The leaves are simple, heart shaped, ovate blade with cordate tuberous base an acuminate apex and an entire margin which grows up to 4cm long. <sup>[2]</sup>

They are used in Sierra Leone as chewing sticks and taken as a purge for colic and stomach pains, and symptoms related with worm-infection and given to new born babies to make them grow rapidly. <sup>[3]</sup> In Ghana the leaves are used as laxatives or rubbed on the joints of small children to

help them walk while in Southern Nigeria the leaves serve as a vegetable for food.

Asthma patients usually chew fresh leaves and this helps to relieve wheezing. Leaves of *Gongronema latifolium* are used as spice or condiment in the diet of nursing mothers, [4] which aids in uterine contractions. [5] Furthermore, [3] reported that *G. latifolium* has anti plasmodial activity. In a study by, [6] it stated that the simultaneous administration of *G. latifolium* with chloroquine(CQ) significantly reduced the effect of CQ by lowering bilirubin, aspartate aminotransferase(AST), alanine aminotransferase(ALT) and alkaline phosphatase(ALP) activities as well as urea and creatinine while the singular administration of chloroquine (CQ) in the low dose, increased enzyme activity which may be due to a leakage of cytoplasmic enzymes into circulation as a result of inflammation of the liver cells. The high magnesium content of *G. latifolium* explains its blood pressure lowering property. [7] The aqueous leaf extract was also reported to express anti-ulcer, anti-pyretic [3] and anti-fungal properties. [8,9] reported that the hypoglycaemic effect of *G. latifolium* can be observed by ingestion of methanolic leaf extract of the leaf with semi-solid meals in alloxan induced diabetic mongrel dogs through mechanism of delayed gastric emptying by gradual release of nutrients for absorption and prevent accumulation of insulin.

The significance of the liver as a principal organ for detoxification of substances cannot be over looked; knowing that failure to carry out this function effectively will have a very harmful effect on virtually every organ of the body. Beside adverse effects from herb itself, adulteration, inappropriate formulation or lack of understanding of plants and drug interaction have led to adverse reactions which are sometimes life threatening or

lethal. [10] Apparently, little research and documentation has been done on the beneficial and adverse effects of this plant on the cellular integrity of the liver and bone marrow hence reason for the choice of these organs.

This study therefore aims at demonstrating the effects (if any) of *Gongronema latifolium* on the histology of the liver and bone marrow.

## MATERIALS AND METHODS

### Experimental Animals

Thirty (30) weaned wister rats purchased from the animal house at University of Jos were used for the study. Animals were allowed to acclimatize for one week. The cages were cleaned daily and disinfected weekly and animals were cared for in accordance with the recommendations provided by National Institutes of Health "Guide for the Care and Use of Laboratory Animals" (NCR, 1985).

### Experimental Design

The thirty (30) weaned Wister rats were randomly divided into three (3) groups of ten (10) rats, each treatment group having 2 replicates of five (5) rats to increase quality assurance and minimize errors. 0.007g/Kg and 0.021g/Kg of extracts were dissolved in 0.1ml of water and administered daily for 21 days to the low and high dose groups. Weight and physical examination of the rats was carried out at interval of one week. Three animals were sacrificed from each group weekly in the course of the study and the liver extracted and fixed immediately in 10% formal saline. The liver was grossed and biopsy taken for histological analysis. The tissues were processed into paraffin block, sectioned at 5um and stained using Harris Haematoxylin and Eosin method. [11] Bone marrow was aspirated from the long bones, smears made, fixed in 95% alcohol and stained using Leishman's staining technique. [12]

Table 1: Groups and treatments given

GROUPS	Control (water ml)	LowDose (extract ml)	HighDose (extract ml)
Group1 (n=5x2)	0.1	-	-
Group2 (n=5x2)	-	0.1	-
Group3 (n=5x2)	-	-	0.1

### Sample Collection and Plant Extraction

Fresh leaves of *G. Latifolium* were procured in August, 2014 from a market in Jos North, Plateau state, Nigeria. They were identified by a taxonomist in the herbarium unit of the Department of Pharmacognosy, Faculty of Pharmaceutical Science, University of Jos. The fresh leaves were handpicked and washed using clean tap water to remove earth materials and dirt. The leaves were air dried under shade for 21 days and mechanically pulverized into coarse powder using mortar and pestle. Aqueous extraction was carried out in the laboratory of molecular biology department, Federal College of Veterinary and Medical Laboratory Technology National Veterinary Research Institute (NVRI) Vom, Nigeria, according to the extraction method described by. [13] Five hundred grams (500g) of *Gongronema latifolium* leaves powder was weighed and soaked in 500ml distilled water, shaken and left for 24hrs. The solution was filtered using No1 Wattman filter paper and filtrate dried in a hot air oven at 35°C for three (3) days to completely get rid of residual water. The dry brown *Gongronema latifolium* extract weighed 43g, and was put in a dried air tight universal bottle and stored in a cool dried place away from sunlight.

### RESULTS

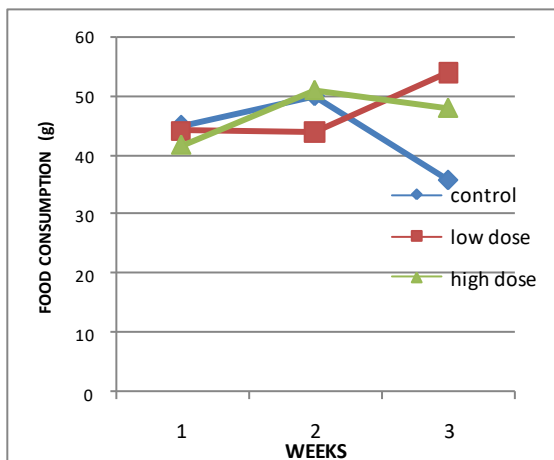


Fig.1.1: Average food consumption (g) of rats in various study groups for three weeks of study

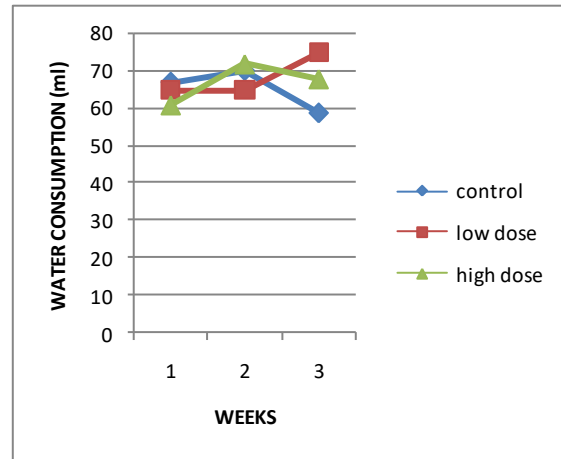


Fig.1.2: Average water consumption (ml) of rats in various study groups throughout the study.

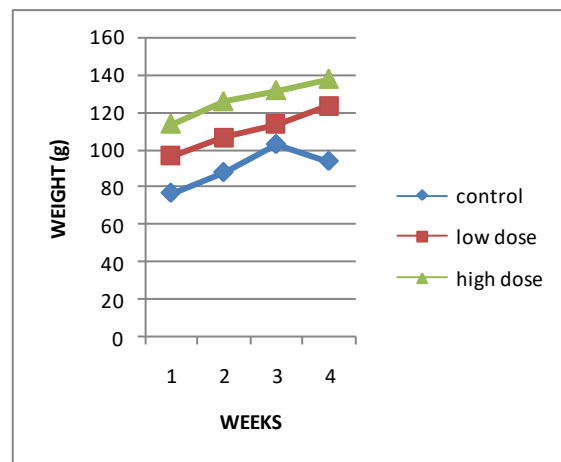


Fig.1.3: Weights (g) of rats in various experimental groups throughout the duration of experiment.

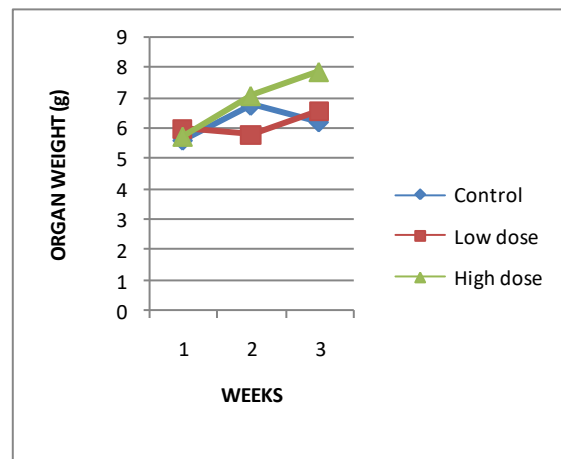


Fig.1.4: Average weight (g) of the liver of rats in various experimental groups throughout experiment.

### Physical Observations.

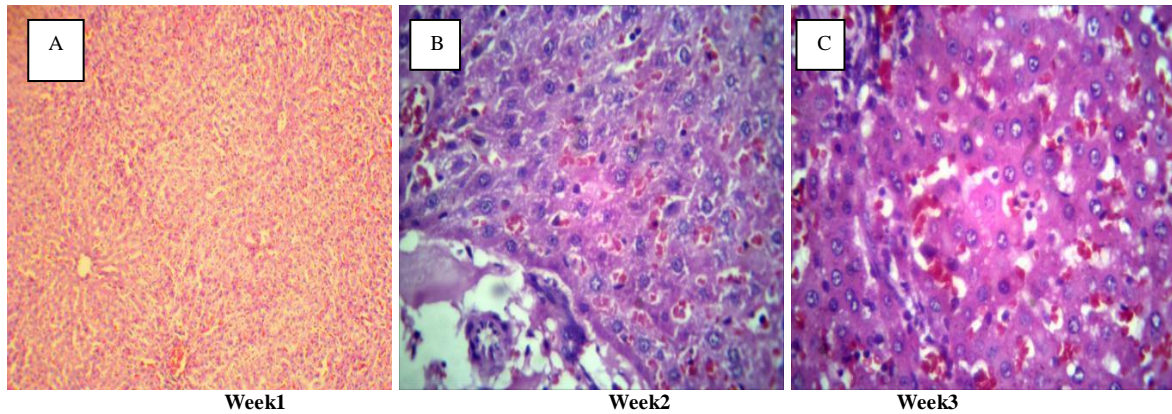
Animals in all groups were seen to be slightly alert and active throughout the study although at the onset of treatment, rats were slightly sluggish. However the low



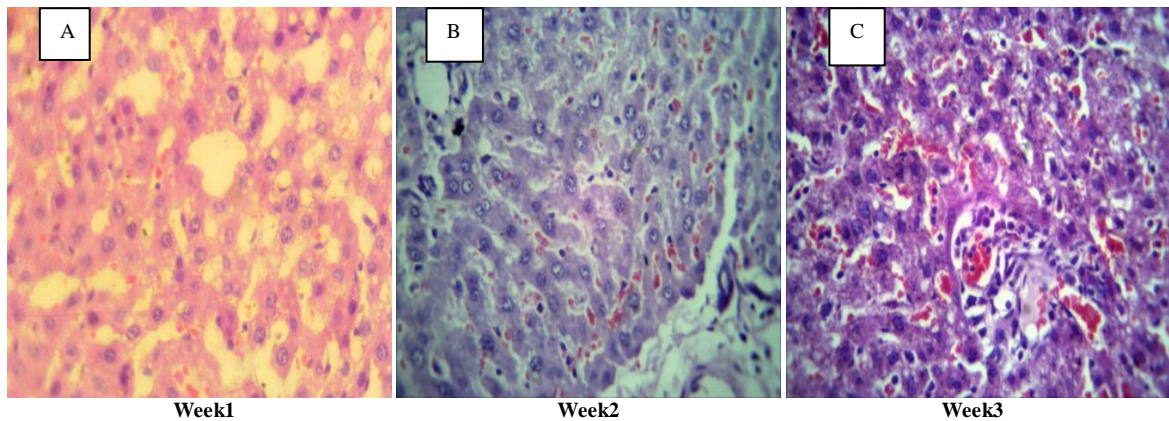
dose group was most active. The fur appearance of experimental rats was smooth all through and they showed no sign of anaemia or jaundice as evidenced by their clear eyes which were checked regularly for signs of paleness or yellow colouration. Their droppings were formed and semi-formed throughout the study.

### Result of Histologic Examination

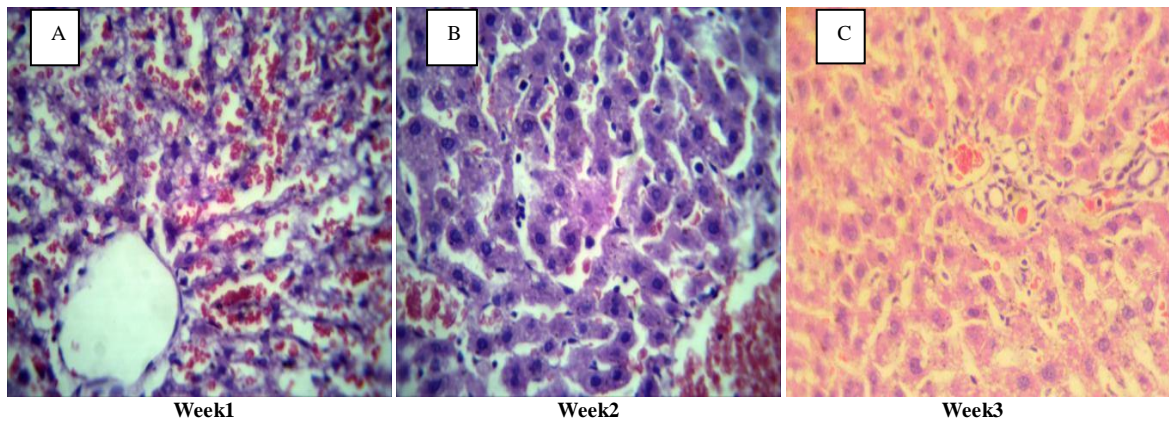
The following photomicrographs were taken from the various treatment groups to present the effect(s) of *Gongronema latifolium* on the liver and bone marrow.



**PLATE 1 (Control):** Section of liver shows cords of hepatocytes separated by sinusoids emerging from a portal vein. Cells clogged with clotted blood. Cells of cytoplasm and prominent vesicular nuclei which are generally unremarkable. (X400 magnification, H and E staining method).

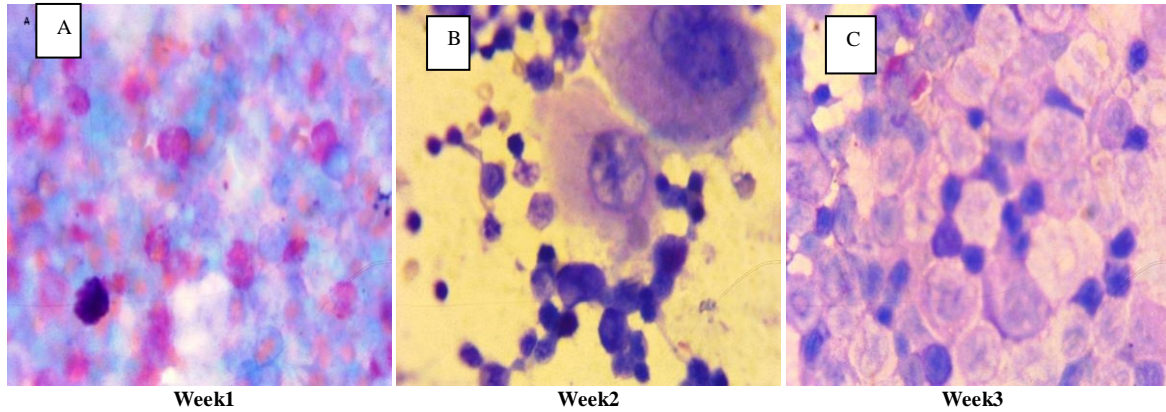


**PLATE 2 (Low Dose-0.007g/Kg):** Sections of liver from low dose in the course of the three weeks showing cords of hepatocytes separated by sinusoids emerging from a portal vein clogged with clotted blood. Histology of liver was generally normal and unaffected by the extract. (X400 Magnification, H and E staining method)



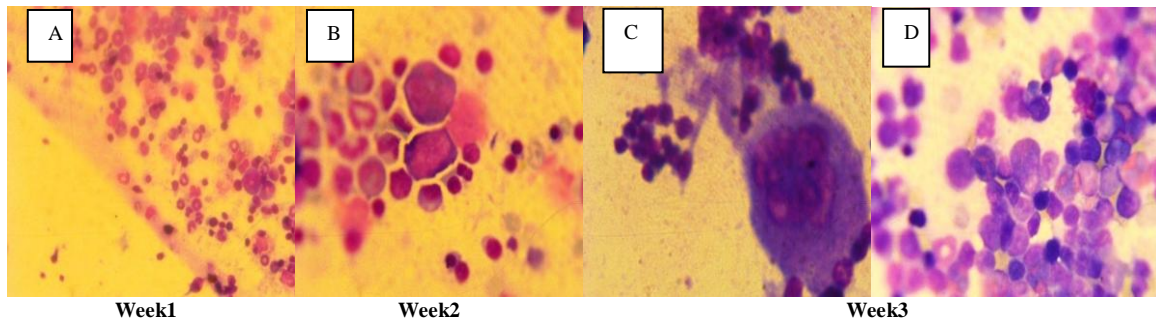
**PLATE 3 (High Dose-0.021g/Kg):** Sections of liver shows cords of hepatocytes separated by sinusoids emerging from a portal vein clogged with clotted blood. Histologic section shows hyperchromatic nuclei of hepatocytes with slight infiltration of polymorphs. (X400 Magnification H and E staining method)





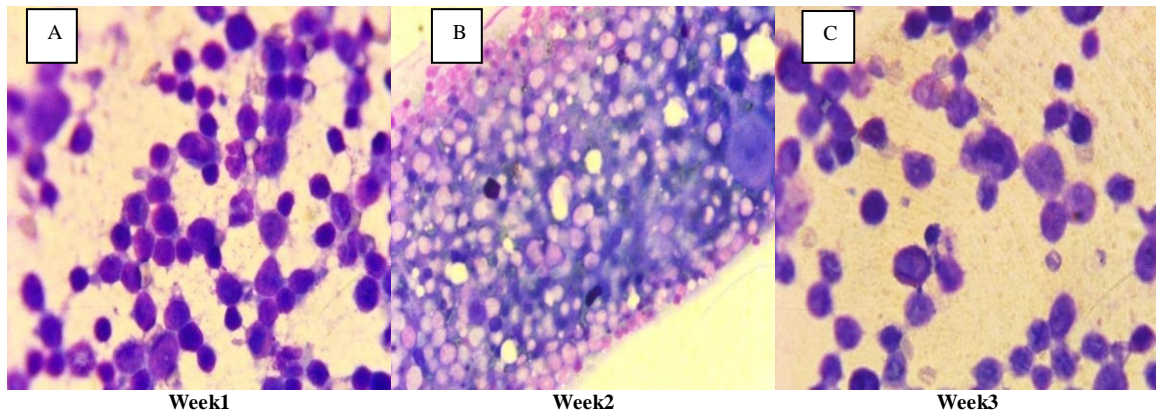
**PLATE 4 (Control)**

- A. Bone marrow film shows numerous lymphocytes and fat cells.
- B. Bone marrow film shows the presence of megakaryocyte. Lymphocytes are the most predominant. Red blood cells are also present.
- C. Bone marrow film shows numerous lymphocytes and adiposites (X1000 Magnification, Leishman's staining method)



**PLATE 5 (Low Dose-0.007g/Kg)**

- A. Bone marrow film shows numerous lymphocytes alongside red blood cells.
- B. Bone marrow film reveals the presence of lymphocytes and megakaryoblasts.
- C. Bone marrow films shows lymphocytes and megakaryoblast.
- D. Bone marrow films show predominance of lymphocytes and red blood cells. (X1000 Magnification, Leishman's staining method)



**PLATE 6 (High Dose-0.021g/Kg)**

- A. Bone marrow film shows lymphocytes with coalescing fat cells.
- B. Bone marrow film shows the presence of numerous fat cells and lymphocytes.
- C. Bone marrow film shows presence of few neutrophils and lymphocytes. (X1000 Magnification, Leishman's staining method)

## DISCUSSION

### Physical observations

At the beginning of experimental study, treated rats were observed to be slightly less active especially when *G. Latifolium* was introduced. The low dose

picked up and became more active than the high dose which was more active. This may be attributed to the beneficial effect of carbohydrates in *G. latifolium* with high energy content. [14] reported that *G. latifolium* has the ability to reduce oxidative

stress. Both groups were more active than the controls at the end of the experiment.

The food consumption pattern of the various groups was presented in figure 1. Control initially increased their food intake and then there was a decrease towards the end of the experiment while the low dose group had relatively stable food consumption and then there was an increase in food intake in the last week. In the high dose group, there was an increase and then a slight decline. Overall, the groups treated with *G. latifolium* appreciated in their food consumption to different degrees except the control in which food consumption at the end was much lower than at inception. The increased food intake in the low dose group could be due to the basic property of *Gongronema latifolium* which has been reported to promote appetite. At the beginning of experiment, treated rats were observed to be slightly less active especially when *G. Latifolium* was introduced. The low dose picked up and became more active than the high dose which was more active. This may be attributed to the beneficial effect of carbohydrates in *G. latifolium* with high energy content. [14] reported that *G. latifolium* has the ability to reduce oxidative stress. Both groups were more active than the controls at the end of the experiment.

The astringent property of tannins [15] could be responsible for decreased feeding in high dose group as was suggested by [9] that *G. Latifolium* when ingested with a solid meal, through the mechanism of delayed gastric emptying, slows digestion due to the presence of saponin. This is also responsible for the decreased food intake in high dose group. Tannins on the other hand has anti-diarrhoea properties which may be responsible for the semi formed and formed droppings of the rats.

The water intake of the rats is presented in figure 1.2, generally followed a pattern similar to their food consumption. This is understandable in view of the fact that the amount of dried food pellets consumed will determine the water consumption.

There was appreciable weight gain in all groups (fig.1.3) respectively. This can be attributed to the plant's appetite promoting effect thereby enhancing growth. [16] had a similar finding and stated that *G. latifolium* may be used to reverse, prevent or reduce weight loss, growth depression and hematotoxicity in diabetic subjects. It is however noted that growth performance of rats can vary due to breed/genotype, age, nutrition, duration of experiment, ambient temperature, disease as well as management.

The fur appearance of all the treatment groups was smooth all through duration of experiment. This may not be attributable to *G. latifolium* alone since the control group had similar appearance. It is however certain that the plant enhance normal physiologic activities thereby improving physical appearance of which fur appearance is not an exception. There was no mortality or morbidity in the entire groups treated with *G. latifolium*.

#### **Histological Observation of the Liver**

The histology of the liver throughout the experiment in the control group and low dose groups were observed to be normal (plates 1 and 2). The normal architecture of the liver was not distorted, cellularity and staining was normal. Histology of the liver was generally normal and unaffected by the dosage and/or duration of administration of extract at low doses which is consistent with the findings. [17]

On the other hand, the high dose group presented evidence of toxicity (plate 3). Although the structure of the liver was maintained and there was slight enlargement of the nuclei of hepatocytes, hyperchromasia and infiltration of inflammatory cells. This may be associated with an immunological response noting that there was also infiltration of polymorphonuclear leucocytes. However, [18] from a biochemical perspective reported that, *G. latifolium* has the ability to reduce the level of liver enzymes in the blood hence has a protective effect on the liver. He suggested that this may be due to the hepa-

protective properties of flavinoids which exerts a membrane stabilizing action that protects the liver cells from injury. This is agreeable with our observations in the low dose group but not applicable to the high dose. There was obvious inflammatory reaction.

#### **Histology of the Bone marrow**

Histology of Bone marrow smears of control, low dose(0.007g/Kg) and high dose(0.021g/Kg) groups show presence of lymphocytes and fat cells (plates 4, 5 and 6). Low dose group has more lymphocytes which may be a reaction to *G. latifolium* and lymphoid cells than the control group. The increased production of these white blood cells was attributed to the innate immunity triggered by the inflammatory process taking place in the liver and related organs initiated by the plant extract. Lymphoblasts present in the low dose group (plate 5A) are known to play a role in hypersensitivity reactions, allergic and inflammatory responses also indicating an immune response to an antigen. Presence of blast cells which are recognizable haemopoietic precursors in the low dose group (plate 5B) indicates an increase in the rate of production of these leucocytes. Megakaryocytes are present in the control and low dose groups (plates 4B and 5C).

Fat cells are seen in control, low dose and high dose groups respectively. In the high dose group, the production of these fat cells was massive with decrease in the production and number of blood cells (reduced bone marrow cellularity). This can result to anaemia which will eventually lead to hypersplenism and enlargement of the liver.<sup>[19]</sup>

The amount of leucocytes and their precursor cells produced in low dose are higher compared to the control group which is even higher than the high dose group. This indicates that there is a serious immunological response which has stimulated production of more leucocytes in low doses while impairing bone marrow cellularity in high doses. Therefore

*Gongronema latifolium* is said to enhance immunity at a low doses.

#### **CONCLUSION**

The histological effects of *Gongronema latifolium* on the liver and bone marrow of Wistar rats investigated highlights marginal enlargement of nuclei of hepatocytes, infiltration of polymorphs, hyperchromatic and dense nuclei reflecting an evidence of hepatotoxicity which suggests a measure of liver damage which could lead to chronic conditions when consumed in doses higher than 0.007g/kgBW. There was no remarkable effect in the low dose group. This is an indication that the effect of the extract is dose dependent.

Simultaneously, the bone marrow is seen to produce more white blood cells (lymphocytes and neutrophils) at low dose of 0.007g/KgBW which is a sign that it enhances innate immunity to inflammation, allergic and hypersensitivity reactions while at high dose of 0.021g/KgBW, it causes anaemia of which with prolong use, could become aplastic and even lethal. Therefore the effect of *Gongronema latifolium* leaf extract being dose dependent is toxic at high doses of about 0.021g/KgBW.

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How to cite this article: Adisa JO, Danladi SY, Egbujo EC et al. Effects of aqueous leaf extract of *gongronema latifolium* on the histology of the liver and bone marrow of wistar rat. *Int J Health Sci Res*. 2017; 7(3):83-90.

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