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Repeated Oral Administration of Febi Super Bitters Correlated with Some Tissue Toxicity in Male Wistar Rat

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Abstract: This study investigated the toxic implication of Febi Super Bitters in Male Wistar rats because of upsurge in its usage for therapeutic reasons. Eighteen rats, with an average weight of 86. 2±4.43 g, were randomly distributed into three equal groups of six rats per group. The rats were acclimatized for 14 days and 0.308 and 0.462 mL kg^{-1} b.wt. of Febi super bitters were administered daily to groups B and C, respectively for 56 days, while group A received distilled water. The toxicity of the herbal bitter was assessed by determining the activities of Lactate Dehydrogenate (LDH), Alkaline Phosphatase (ALP) and Acid Phosphatase (ACP) in the liver, kidney, small intestine, heart, brain, lungs, spleen, serum and histological studies on the organs. The activities of LDH were significantly reduced ($p<0.05$) in the liver, small intestine and lung, while it increased significantly ($p<0.05$) in the brain and serum. ALP activities decreased significantly ($p<0.05$) in the liver, kidney, small intestine and heart but increased ($p<0.05$) in brain, spleen and serum. ACP activities in the liver and kidney were decreased ($p<0.05$) and increased significantly ($p<0.05$) in the spleen and serum. Increases were significantly recorded in serum total bilirubin, unconjugated bilirubin, total protein and globulin, while reductions were recorded in the conjugated bilirubin and A/G ($p<0.05$). Histoarchitecture of the lungs, spleen and small intestine revealed marked cellular distortions. In the light of these findings, the habitual consumption of Febi super bitters is not recommended as it has underlined toxicity in some vital internal organs.

Key words: Upsurge, usage, febi super bitters, habitual, toxicity, internal organs

INTRODUCTION

Recently, alternative therapies are now a major component of the over the counter market in many developed and developing countries. Due to some perceived deficiencies in orthodox pharmacotherapy, natural remedies or herbal supplements are commonly employed in developing countries for the treatment of various diseases (Zhu *et al*., 2002). In developing countries such as, Nigeria, due to low standard of living, low doctor-patient ratio and extended waiting time before the doctor is accessed, people hardly visit the hospital for medical check-up unless they feel ill or the disease conditions become obvious that self medication cannot contain anymore. Herbal bitters are hence very popular medicine for keeping healthy in many homes in Nigeria, of which Leon bitters, Yoyo bitters, Kaka bitters, Febi super bitters and Daily living bitters are the most common of the locally available herbal bitters in the southwest geopolitical zone in Nigeria. Therefore, these herbal bitters are consumed daily by both young and old because of the alleged claims by their manufacturers on the beneficial effects of their usage such as, detoxification of the blood, weight control, indigestion, restoration of youthfulness, insomnia, skin allergies, anti-inflammatory, modulation of the immune system, management of degenerative diseases among others (Blumenthal, 1989; Claff, 2003). The principal constituents in most herbal bitters are secondary plant metabolites such as alkaloids, flavonoids, polyphenols among others, suspended in water or alcohol (tincture).

The upsurge in the usage of these herbal bitters or products, as a ready alternative to turn to for various medical conditions is enhanced by the long history and belief that herbal products are natural and safe (Ogbonnia *et al*., 2011). In Nigeria, it is gradually becoming acceptable that herbal supplement or products are efficacious and safe, as a vast of the products are

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alleged to be recognized with approval numbers on their label from the national regulatory body on food and drug administration, National Agency for Food Drugs Administration and Control (NAFDAC). It is expedient however, due to the upsurge in the use of these herbal preparations and the rationale for their utilization, which is based largely on long-term clinical usage that thorough scientific investigations of the toxic implications will go a long way in validating their usage. Thus, the harm that could be associated with the extended usage of such phyto-medicines requires that the practitioners be informed of the reported incidence of tissues toxicity emanating from ingestion of herbal products (Tedong *et al*., 2007). Therefore, the aim of this research work was to assess the toxic implication of the sub-chronic administration of Febi super bitters in male Wistar rats.

MATERIALS AND MATERIALS

Materials

Herbal bitters: Febi super bitters was a product of Dominion Nigeria International, 2 Victory Street, Iba Tedo, Ojo, Lagos. Nigeria.

Quantitative assay kits and other reagents: Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), total protein, albumin and bilirubin concentrations were products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain.

Other reagents: The chemicals and reagents utilized in the study were of analytical grade and were purchased from the British Drug House (BDH) Poole England and Sigma Aldrich Chemical Co. Inc., Milwaukee, Wis., USA.

Laboratory animals: Eight to ten weeks old male Wistar rats of average body weight of 86.2±4.43 g were obtained from the Animal Care Facility II, Ladoke Akintola University of Technology (LAUTECH), Osogbo, Osun State. The rats were fed with rat pellet (product of Bendel Feeds and Flour Mills Ltd, Ewu, Edo State, Nigeria).

Experimental animals and procedure: The eighteen male Wistar rats were randomly grouped into three, comprising of six rats per group. The rats were housed in cages made of wooden frames and metal netting and were fed *ad libitum* with rat pellet and tap water with 12 h light/dark cycle. The cages were cleaned every morning and disinfected at intervals of 3 days. The rats were allowed to acclimatize for 14 days before herbal bitters administration was commenced. Calculated doses of the Febi super bitters in mL kg^{-1} b.wt. as instructed in the manufacturer's pamphlet were administered to male Wistar rats as illustrated:

Group A: Control, received 1.0 mL distilled water
Group B: Received 0.308 mL kg^{-1} b.wt. of Febi super bitters (FB)
Group C: Received 0.462 mL kg^{-1} b.wt. of Febi super bitters (FB)

Administration of the herbal bitters was performed orally once daily between 7:20 pm±30 min, using metal cannula attached to a 1.0 mL syringe. Administration lasted for 56 days, after which the rats were fasted for 12 hand the organs of interest (brain, liver, kidney, small intestine, heart, spleen and lung) were exercised, cleansed and blotted with filter paper. The study was conducted in accordance with the regulation of the National Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH, 1985).

Determination of enzyme activities: The lactate dehydrogenase (LDH) activity was determined by pyruvate kinetic liquid reaction, according to the method described by Murray and Hartmann (1985). The activity of alkaline phosphatase (ALP) was determination by p-Nitrophenylphosphate kinetic reaction, according to the method described by Tietz *et al.* (1995). The activity of acid phosphatase (ACP) was determined by α-Naphtyphosphate kinetic reaction, according to the method described by Abbort *et al.* (1984). The serum and tissue homogenates were stabilized with 50 μL of acetic acid (R_4) per mL of sample.

Serum proteins determination: The determination of serum total protein concentration was determined by Biuret colourimetric reaction, according to the method as described by Burtis *et al.* (1999), while serum albumin concentration was determined by bromocresol green colourimetric reaction, according to the method as described by (Gendler, 1984). Globulin concentration was obtained by the formulae:

$$\text{Globulin} = \text{Total protein} - \text{Albumin}$$

Serum bilirubin determination: The serum bilirubin levels were determine by Dimethylsulphoxide (DMSO) colourimetric reaction, according to the method as described by Kaplan *et al.* (1984).

Histology: The histological studies were performed on liver, kidney, brain, heart, spleen, small intestine and lung following the procedure described by Krause (2001).

Statistical analysis: This research work employed a completely randomised design (CRD) model and the results were expressed as Mean±SEM of 5 determinations. Results were analyzed using Statistical Package for Social Sciences (SPSS) 16.0 for Window software. Results were subjected to one way Analysis of Variance (ANOVA) to test the effect of each dose level on the parameter under investigation at 95% level of confidence. The Duncan Multiple Range Test (DMRT) was conducted for the pair-wise mean comparisons, to determine the significant treatment dose at 95% level of confidence. Values were considered statistically significant at ($p<0.05$) and denoted by different alphabets (Mahajan, 1997).

RESULTS

Enzyme activities: The result obtained in the activities of enzymes determined in the male rats is depicted in Table 1. The administration of Febi super bitters produced significant reductions ($p<0.05$) in the Lactate Dehydrogenase (LDH) activity in liver, small intestine and lungs in male rats (Table 1). In the heart, brain and serum of male rats administered with Febi super bitters, significant increases ($p<0.05$) were recorded in the LDH activity. The activity of Alkaline Phosphatase (ALP) was decreased significantly ($p<0.05$) in the liver, kidney and small intestine of rats administered the herbal bitters in a dose dependent manner. However, ALP activity increased significantly ($p<0.05$) in the brain, lungs and spleen in rats administered Febi super bitters. At 0. 462 mL kg^{-1} b.wt. of the herbal bitters, significant decrease and increase ($p<0.05$) were recorded in the ALP activity, respectively in the heart and serum in male rats. A dose dependent significant decrease ($p<0.05$) was obtained in the acid phosphatase (ACP) activity in the liver and kidney of rats administered with the herbal bitters. In the spleen, the activity of ACP was markedly increased ($p<0.05$) in the rats administered with 0. 462 mL kg^{-1} b.wt. of Febi super bitters. Nonetheless, a dose dependent significant increase ($p<0.05$) was obtained in the ACP activity in the serum of male rats administered with the bitters (Table 1).

Table 1: Effect of Febi super bitters on the activities of some enzyme markers in male rat

Activity (IU L^{-1})	Control	Doses (BW) 0.308 (FB)	0.462 (FB)
LDH			
Liver	189.13±70.51^a	146.43±60.62^b	131.44±5.77^b
Small intestine	460.34±13.65^a	255.60±10.40^b	155.05±6.10^c
Heart	189.55±60.65^a	201.25±90.54^b	140.25±7.75^c
Brain	220.15±90.65^a	300.50±50.94^b	240.35±8.15^c
Lung	425.45±12.67^a	215.25±12.35^b	232.84±9.75^c
Serum	62.65±50.34^a	101.67±4.91^b	260.50±9.15^c
ALP			
Liver	141.04±40.11^a	121.37±50.21^b	92.19±5.26^c
Kidney	169.31±50.51^a	155.35±60.24^b	101.93±5.71^c
Small intestine	180.34±90.93^a	225.74±50.57^b	114.95±5.25^c
Brain	202.15±70.28^a	137.65±40.75^b	231.44±5.54^b
Lung	95.45±30.85^a	179.31±40.65^a	161.35±3.95^c
Heart	187.20±60.17^a	194.55±40.15^b	161.35±6.54^b
Spleen	165.95±50.64^a	119.75±30.55^a	187.95±5.25^b
Serum	115.56±30.35^a	255.60±10.40^b	132.85±4.05^c
ACP			
Liver	235.37±11.26^a	189.55±80.68^b	162.23±8.67^c
Kidney	352.44±70.54^a	319.22±70.42^b	275.83±8.41^c
Spleen	178.34±90.35^a	182.37±60.35^a	221.75±7.45^b
Serum	87.35±40.25^a	220.55±11.45^b	253.65±8.75^c

Values are Means±SEM, n = 5, Values bearing different alphabets are significantly different ($p<0.05$), Key: BW: (mL kg^{-1} b.wt., FB: Febi super bitters

Liver function indices: The data of the liver function test following the administration of Febi super bitters in rats is presented in Table 2. Febi super bitters at 0. 462 mL kg^{-1} b.wt. of increased significantly ($p<0.05$) the concentration of the total bililrubin, while serum total protein concentration was increased significantly ($p<0.05$) dose dependent. The concentration of conjugated bilirubin and the albumin/globulin ratio were decreased significantly ($p<0.05$) at both doses of the herbal bitters (Table 2), while unconjugated bilirubin and globulins concentration were likewise increased significantly ($p<0.05$). The serum albumin concentration was unaltered significantly ($p<0.05$) following the administration of the herbal bitters (Table 2).

Histology: The photo-micrographic representations of the organs were classified as percentages of inflammation or compromise to the integrity of cells in the captured area of the organs, in which <25% is non-significant and >25% is significant. Febi super bitters at 0. 462 mL kg^{-1} b.wt. only compromised the structure and histoarchitecture of the hepatocytes as revealed in the photomicrographs of the liver of rats (Fig. 1-3). Paradoxically, the administration of the Febi super bitters to rats at the different doses did not reveal photographically any tissue damage in the kidney

Table 2: Liver function indices in Wistar rats administered with the herbal bitters

Concentration (mg dL^{-1})	Control	Doses (BW) 0.308 (FB)	0.462 (FB)
Total bilirubin	37.44±2.11^a	38.22±2.05^a	43.05±2.16^b
Conjugated bilirubin	28.88±1.67^a	22.46±2.18^b	22.94±2.42^b
Unconjugated bilirubin	10.47±0.89^a	14.66±1.67^b	19.02±2.16^b
Total protein	5.72±0.82^a	6.96±0.43^b	7.15±0.37^c
Albumin	3.22±0.90^a	3.18±0.55^a	3.01±0.41^a
Globulin	2.67±0.16^a	4.02±0.44^b	4.05±0.27^b
A/G	1.08±0.28^a	0.85±0.19^b	0.88±0.11^b

Values are Mean±SEM; n = 5, Values bearing different alphabets are significantly different ($p<0.05$), Key: BW (mL kg^{-1} b.wt.), FB: Febi super bitters)

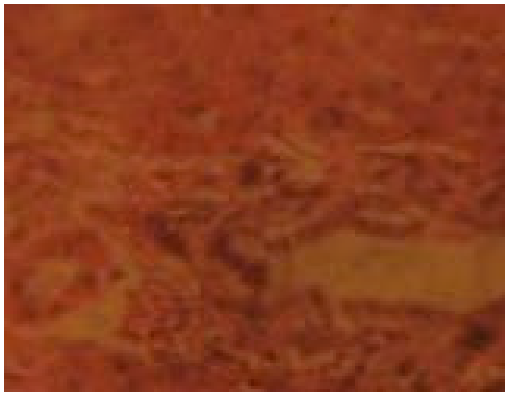

Fig. 1: Photomicrograph of liver of male rat administered distilled water (Mag×100; H and E) (A normal liver)

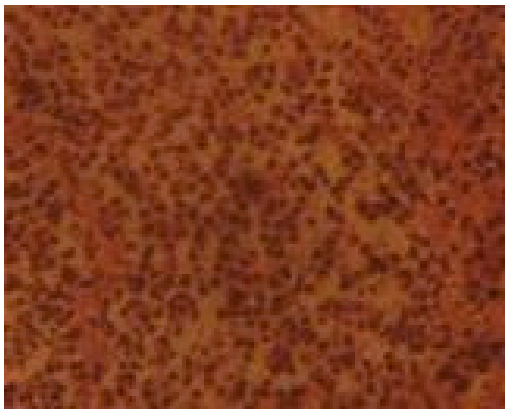

Fig. 2: Photomicrograph of liver of rat administered with 0. 308 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A liver with non-significant hepatocytes enlargement)

(Fig. 4-6). However, dose dependent significant alterations were revealed in the photomicrographs of the spleen of rats administered the herbal bitters, compared to the control (Fig. 7-9). The sinosoids and lobes (lymphoid tissues) were enlarged and the red pulps were degenerated with the infiltration of the white pulp (Fig. 7-9). The photomicrographs of the small intestines showed that the administration of the herbal bitters compromised the structure and histoarchitecture of the small intestines, in which the lymphoid tissues were enlarged and aggregated, thereby eroding of the villi and lysing of the epithelia cells (Fig. 10-12). The administration of the herbal bitters did not reveal any compromise in the histoarchitexture of the brain (Fig. 13-15), heart (Fig. 16-18) and lungs (Fig. 19-21).

Fig. 3: Photomicrograph of liver of rat administered 0. 462 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A liver with significantly enlarged central vein with haemorrhage)

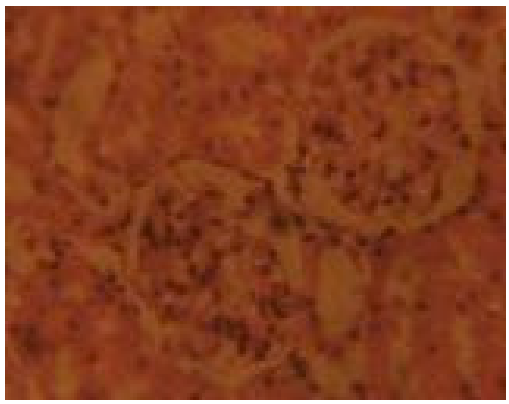

Fig. 4: Photomicrograph of kidney of male rat administered distilled water (Mag×100; H and E) (A normal kidney with non-significantly enlarged cells at the connective tissues)

DISCUSSION

Phyto-medicines or natural supplements are commonly used in developed and developing countries for the treatment of various diseases, which is being an alternative therapy to compensate for some perceived deficiencies in orthodox pharmacotherapy (Zhu *et al*., 2002). However, the safety of the use of herbal medicine or products has recently been questioned due to reports of illness and fatalities (Veiga-Junior *et al*., 2005; Park *et al*., 2010); hepatotoxicity (Saad *et al*., 2006) and nephrotoxicity (Debelle *et al*., 2008). The measurement of the activities of 'marker' or diagnostic enzymes in tissues

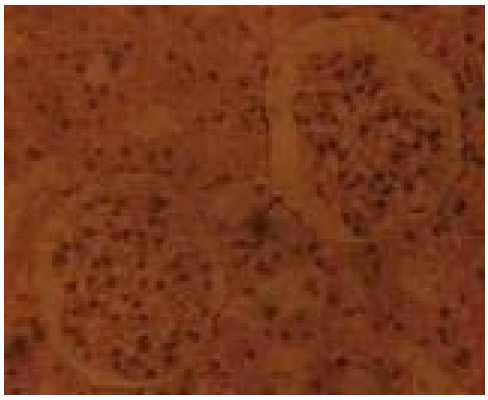

Fig. 5: Photomicrograph of kidney of rat administered with 0.308 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal kidney)

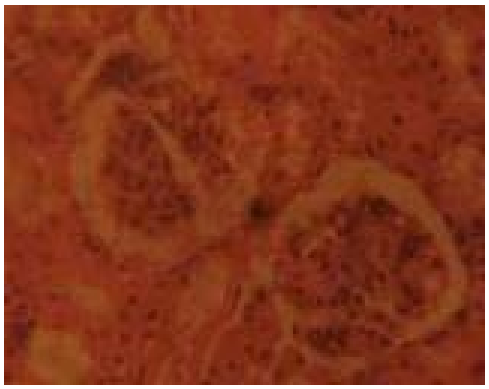

Fig. 6: Photomicrograph of kidney of rat administered 0.462 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal kidney)

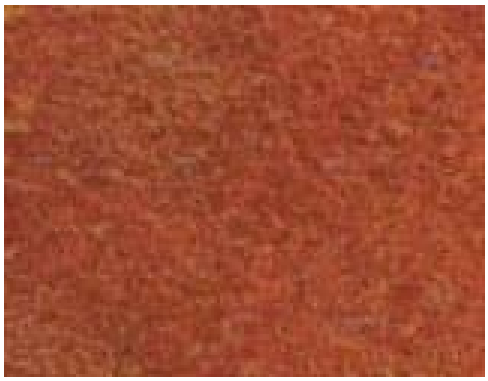

Fig. 7: Photomicrograph of spleen of male rat administered distilled water (Mag×100; H and E) (RP red pnlp) (A spleen with non-significant increase in the red pulp)

Fig. 8: Photomicrograph of spleen of rat administered with 0.308 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A spleen with significantly enlarged sinosoids and lobes and red pulp degeneration)

Fig. 9: Photomicrograph of spleen of rat administered with 0.462 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A spleen with pigments and respective significant increase and decrease in white pnlp and red pulp)

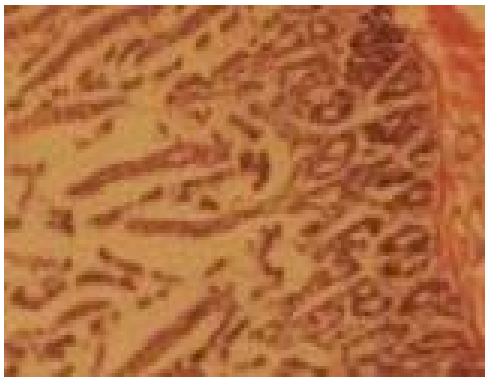

Fig. 10: Photomicrograph of small intestine of male rat administered distilled water (Mag×100; H and E) (A normal intestine with non significantly enlarged lymphoid tissues and separated gastric pith)

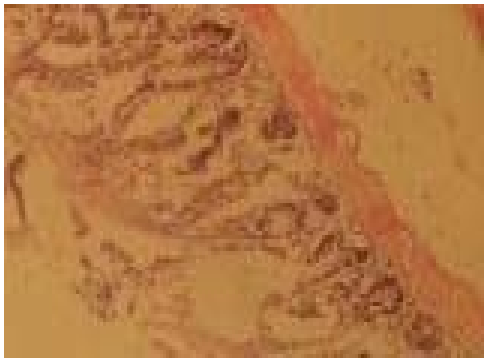

Fig. 11: Photomicrograph of small intestine of rat administered with 0. 308 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (Intestine with significantly increased lymphoid tissues and cell lysis)

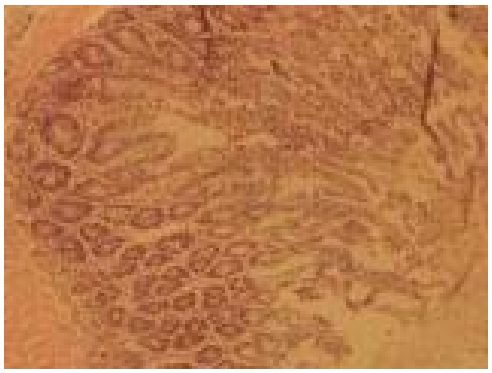

Fig. 12: Photomicrograph of small intestine of rat administered with 0.462 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (Intestine with significantly increased and prominent lymphoid tissues and eroded villi)

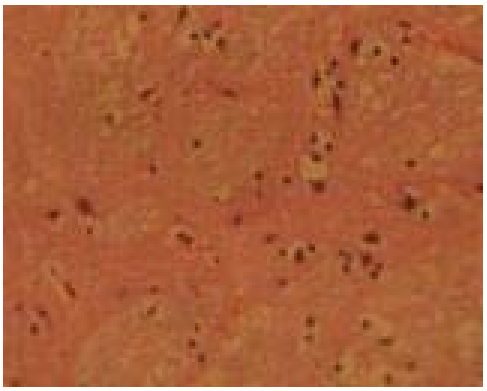

Fig. 13: Photomicrograph of brain of male rat administered distilled water (Mag×100; H and E) (A normal brain)

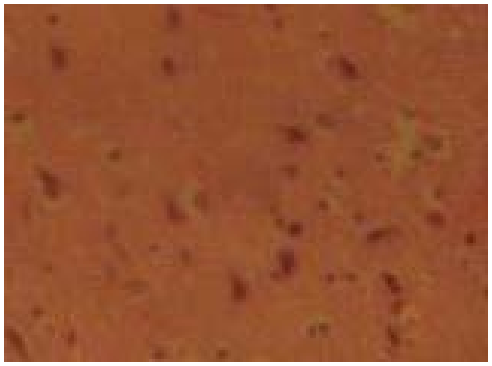

Fig. 14: Photomicrograph of brain of rat administered with 0.308 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal brain)

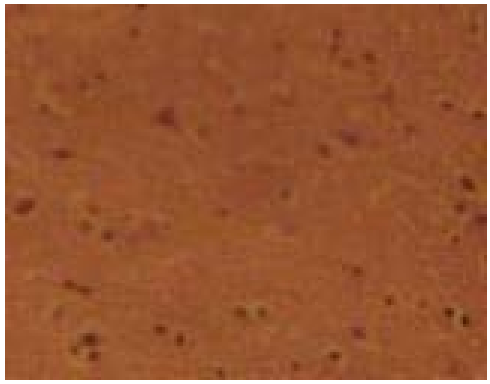

Fig. 15: Photomicrograph of brain of rat administered with 0.462 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal brain)

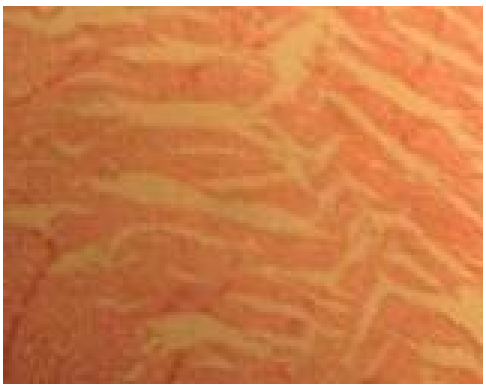

Fig. 16: Photomicrograph of heart of male rat administered distilled water (Mag×100; H and E) (A normal heart)

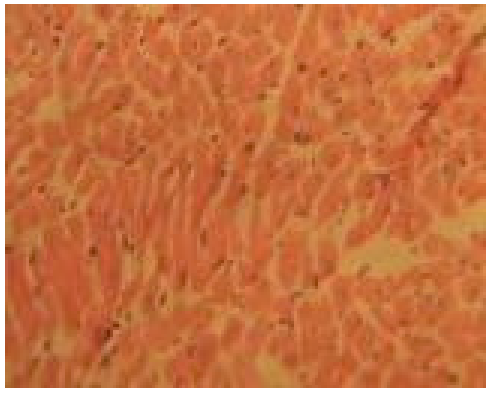

Fig. 17: Photomicrograph of heart of rat administered with 0. 308 mL kg^{-1} mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal heart)

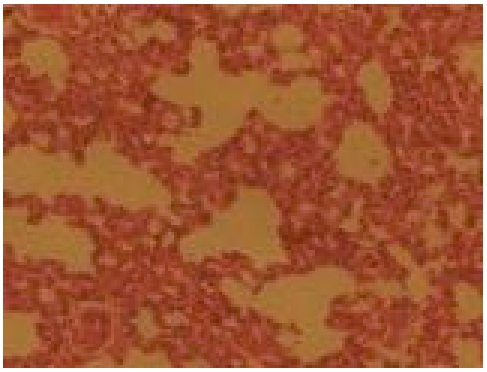

Fig. 20: Photomicrograph of lungs of rat administered with 0. 308 mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal lungs)

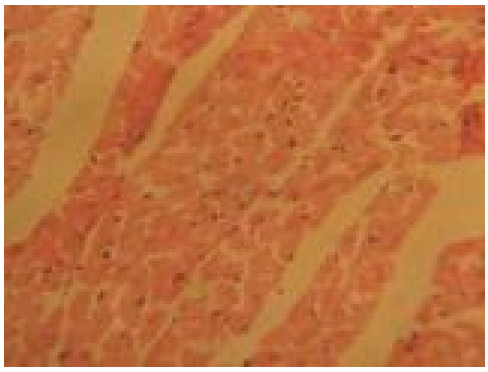

Fig. 18: Photomicrograph of heart of rat administered with 0. 462 mL kg^{-1} mL kg^{-1} b.wt. of Febi super bitters (Mag×100; H and E) (A normal heart)

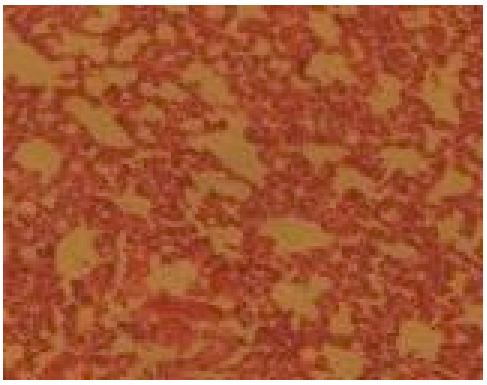

Fig. 21: Photomicrograph of lungs of rat administered with 0. 462 mL kg^{-1} b.wt. of Febi super bitters (Mag×s100; H and E) A normal lungs

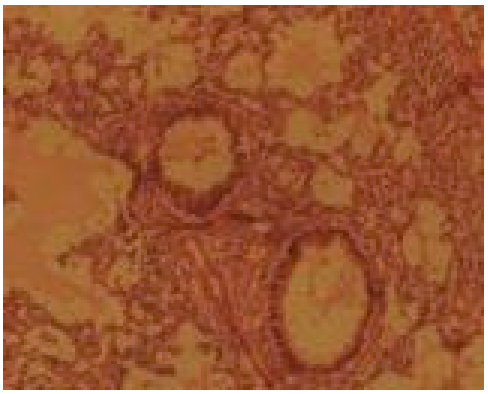

Fig. 19: Photomicrograph of lungs of male rat administered distilled water (Mag×100; H and E) (A normal lungs)

and serum plays a key role in clinical diagnosis, clinical investigations and the assessment of toxicity/safety risk of xenobiotics (Radhika *et al*., 2012). The activities of the enzymes determined in this study are good markers of tissue cytolysis and or, damage to the plasma membrane.

In this study, the decreased activity presented in Alkaline Phosphatase (ALP) in the liver, kidney, small intestine and heart (Table 1) indicated that the administration of Febi super bitter lead to the inactivation of the enzyme *in situ* or the distortion of the plasma membrane, which could have resulted in the release of ALP into extra-cellular spaces. ALP is ubiquitous in nature, due it main role in the provision of inorganic phosphate for cell growth by the hydrolysis of exogenous phosphate esters, which can't cross the cytoplamic membrane (Rao and Rao, 2001). Therefore, such reductions in the ALP activities would hinder the

adequate transportation of needed ions or molecules across their cell membranes, which may lead to starvation of the cells (Akanji *et al*., 1993) and might also, adversely affect other metabolic processes where the enzyme is required, such as the synthesis of nuclear proteins, nucleic acids and phospholipids as well as in the cleavage of phosphate esters among others (Oyewo *et al*., 2012). In addition, in the liver, if the hepatocytes, bile ducts or gall bladder system is/are not functioning properly or are blocked, ALP is not excreted through the bile but is released into the blood stream. Manjunatha *et al*. (2005) reported that ALP activity is related closely to the functioning of hepatocytes and any alterations in its activity may be due to a challenge or exerted pressure on the liver.

In the brain lungs and spleen of male rats administered Febi super bitters, the increase in ALP activity might be due to increased functional activity that probably led to *de novo* synthesis of the enzyme molecules *in situ*. However, the hyper-activity of ALP could constitute a threat to the survival status of the cells that are always dependent on a variety of phosphate esters for their vital processes. Hyper-activity of ALP may lead to indiscriminate hydrolysis of phosphate ester metabolite of the tissues, which is thus, an important biochemical symptom of cytolysis. Consequently, this may adversely affect the facilitation of the transfer of metabolites across the cell membrane (Yakubu *et al*., 2005). The concomitant increase in the serum ALP activity at 0. 462 mL kg^{-1} b.wt. of the herbal bitters complement the ALP released from injured tissues, such as the liver, kidney, small intestine and heart (Table 1).

The decrease in acid phosphatase activity following the administration of Febi super bitters indicate tissue injury in the liver and kidney that might be due to inflammatory responses, which increased the activities of the lysosome in the tissues. The increase in the ACP activity in the spleen might denote an increase in the need for active transport in the membranes (Shahjahan *et al*., 2004), which could have led also to loss of other proteins (Akanji *et al*., 1993), as indiscriminate hydrolysis of phosphate esters might occur. The indiscriminate hydrolysis of phosphate esters, which are the potential energy sources for the cells, may suggest a possible threat to the energy status of the organs as this might result in autolysis and consequently cell death. The trend in the serum ACP activity is a strong indicator of tissue injury that could have arose from inflammatory responses in tissues and/anemia, which may be due the phyto-constituents in Febi super bitters.

Lactate dehydrogenase (LDH) is cytosolic enzyme that is involved in the regulation of biochemical reactions in the body tissues and fluids (Shinde and Goyal, 2003). The decrease recorded in the LDH activity in the liver, small intestine, heart and lungs (Table 1) indicated tissue injury, blood flow deficiency (ischemia) or death of cells of these tissues. However, a decrease in the activity of LDH that is simultaneous with decrease in the activity of ALP in the same tissues, is quite understandable since LDH is in close proximity to the plasma membrane (Philip, 1995). The reduction in LDH activity may also indicate a drop in endogenous glucose production and the inhibition of endogenous cholesterol synthesis (Jorda *et al*., 1982). The increase in the LDH activity in the brain supported the result of the ALP activity in the brain, which might be due to an increase in functional activity or size. The markedly increased LDH activity in the serum of rats following the administration of Febi super bitters supported the indicated injury to the tissues whose LDH activity were decreased and may also, indicate anemia, supporting the result of serum ACP activity. The overall result of the enzyme activity supported the previous report by Oyewo *et al*. (2013) that the sub-chronic administration of Febi super bitters triggered inflammatory responses by the induction of TNF-α and IL-6 in male rats.

Protein profile and bilirubin concentrations in the serum are often used to assess the functional state of the liver and indicate or streamline the type of damage in the liver (Oyewo and Akanji, 2011). Sub-chronic administration of Febi super bitters interfered with the integrity of the liver as indicated by the result of the liver function indices, except albumin (Table 2). Serum total proteins concentrations can reflect nutritional status and may be used to screen or diagnose or monitor patients with kidney and liver diseases (Thierry *et al*., 2011). The increase in the serum total protein levels may indicate inflammation, dehydration, respiratory distress or haemolysis, as decrease in serum total protein are reported in these clinical conditions (Ganong, 2002). This observation is supported by the result of the serum globulin levels, which is known to increase due to the stimulation of the differentiation and proliferation of B lymphocytes. The result of the serum total protein and globulin are in agreement with the previous study by Oyewo *et al*. (2013), in which the administration of Febi super bitters was reported lead to the induction of inflammatory cytokine in rats. Although, as the serum albumin concentrations were not affected by the administration of the herbal bitters, then the decrease the albumin/globulin index was due to the increased levels of globulin in the rats administered with Febi super bitters.

Administration of Febi super bitters to rats led to an imbalance in haem metabolism and excretion at

0. 462 mL kg^{-1} b.wt., which resulted to the increase in the serum total bilirubin level (Table 2). Thus, it can be inferred that the administration of Febi super bitters at the dose resulted in anaemia or increased red blood cell degradation, biliary stricture and/chronic inflammation in the liver. The decrease in the concentration of conjugated bilirubin in the rats administered with the herbal bitters showed that the herbal bitters could not have induced biliary obstruction and hepatotoxicity in the rats, thereby negating the possibilities of the development of obstructive jaundice and hepatocellular jaundice (Vasudevan and Sreekumari, 2000; Champe *et al*., 2005). However, the reported increase in the concentration of unconjugated bilirubin in the rats administered with the herbal bitters indicated haemolytic anaemia or jaundice. This is supported by the result of the serum total bilirubin concentration at 0. 462 mL kg^{-1} b.wt. of Febi super bitters.

Histopathological examination of tissues could serve as complementary evidence to enzyme studies towards revealing any distortion/damage to the normal histo-architexture of the cells. The incidence of congestion of cells displayed by the liver following administration of Febi super bitters at 0. 462 mL kg^{-1} b.wt. (Fig. 3), as evidenced by the enlarged central vein with haemorrhage supported the results of the 'enzyme markers' that were assayed in the liver and those of the liver function tests (Table 1, 2, respectively). The alteration in the histo-architectures of the spleen in rats administered with the herbal bitters (Fig. 8, 9) supported the *in situ* induction of the activity of the 'enzyme marker' assayed in the spleen and also supported the hemolytic anemia indicated by the result of serum bilirubin concentration and the previous report of Oyewo *et al*. (2013) on the sub-chronic use of the Febi super bitters.

The observation in photomicrographs of the small intestine supported the result of the 'enzyme markers', indicating that the sub-chronic use of Febi super bitters eroded the walls of small intestine (Fig. 11, 12). Although, the removal of excess mucus and pile from the gastrointestinal wall is the key mechanism for the expulsion of waste and toxins by herbal bitters needed for the enhancement of the digestion of ingested food substances. However, the sub-chronic use of Febi super bitters might have over-washed the gastrointestinal wall and might have affected the digestion and absorption of food nutrients. This result, therefore, supported the previous report by Oyewo *et al*. (2013) that the sub-chronic administration of Febi super bitters altered the metabolism of glucose and lipids in male rats and suppressed their feed intake, respectively.

On the other hand, the normal architecture revealed in the photomicrographs of the kidney, brain, heart and lungs is an indication that the herbal bitters may not have major adverse effect on these tissues. Thus, the observed increases in the activity of the "marker enzyme' in some cases might actually be artefactual in nature. This could be likened to a similar report by Salazar *et al*. (1998), in which there were arbitral significant differences in the activities of some 'enzyme marker' in the tissue but obvious compromise were seen in the photomicrographs of the such tissues. Although, the observations may be due also to the fact that the compromise or damage in these tissues was still ongoing and thus, might not be obvious yet in the photomicrograph of the tissues. Akanji *et al*. (1993) reported that the onset of most tissues injury when challenged by xenobiotics is seen initially in the alterations of the activities of tissue 'enzyme markers', before been revealed in the histoarchitexture of the tissue.

CONCLUSION

The sub-chronic administration of Febi super bitters resulted in the induction of inflammation in some tissues and haemolysis in male Wistar rats. The daily use of this herbal bitters is discouraged.

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