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Sub-chronic Administration of Febi Super Bitters Triggered Inflammatory Responses in Male Wistar Rats

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Febi super bitters is one the most reported herbal products consumed as blood cleansers, detoxifier, regulator of blood sugar and body weight, pain reliever, immunomodulator etc in the southwest region of Nigeria. Thus, the immunomodulatory activity of the sub-chronic oral administration of Febi Super Bitters was assessed in male Wistar rats. Eighteen rats were randomly picked into three equal groups with an average body weight of 86.2±4.43 g. The rats were acclimatized for 14 days, after which 0.308 and 0.462 mL kg^{-1} body weight of Febi Super Bitters were administered daily to groups B and C respectively for 56 days, while group A received distilled water. Immunomodulatory activities were evaluated by the feed intake, body weight, blood glucose, interleukin 2 (IL-2), interleukin 6 (IL-6), tumour necrosis factor alpha (TNF-α), haematological parameters, serum lipid profile and uric acid, liver reduced glutathione and malodialdehyde. The feed intake, the body weight and the blood glucose concentrations were markedly reduced ($p<0.05$) dose dependently. Serum concentrations of IL-2 were unchanged ($p>0.05$), but IL-6 and TNF-α were altered significantly ($p<0.05$). Significant decreases were recorded in the erythrocyte parameters and serum lipids, except in high density lipoprotein- and very low density lipoprotein-cholesterol. Leukocytes, platelets and uric acid levels were increased ($p<0.05$). The sub-chronic use of Febi super bitters indicated the induction of oxidative stress and inflammatory responses that possibly stimulated the immunoactivities. From the foregoing, the alleged daily consumption of Febi super bitters as blood tonic or immunomodulatory agent is not recommended.

Key words: Febi super bitters, immunomodulatory, inflammatory responses, daily use

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INTRODUCTION

The origin of herbal bitters has been traced back to more than 5,000 years, possibly due to the opening of trade routes with China (Saad *et al*., 2006). The recent craze for herbal bitters was reported to be due to the proven efficacies of some herbal preparations and have resulted in a great patronage for many products that comes with the name "herbal" (Ogbonnia *et al*., 2011). The major constituents of most of these herbal bitters are secondary plant metabolites such as alkaloids, saponins, flavonoids, polyphenols among others, suspended in water or alcohol (tincture).

The discovery that some substances derived from plant sources are potential therapeutic against various human diseases and the alleged nutritional values of such plants, have made vast a number of plants invaluable and indispensable to human (Steve *et al*., 2008). With the recent escalation in the patronage for many products that comes with the name "herbal bitters or supplements", Febi Super Bitters is one of the popular herbal bitters in the in the south-west region of Nigeria. Others in the list include Leon bitters, Yoyo bitters, Kaka bitters, Daily living bitters, Enis bitters etc. These herbal bitters have become a popular medicine for keeping healthy in many homes in Nigerian and their manufacturers in an aggressive marketing drive, claim they are recipes for indigestion, weight control, detoxifiers, blood purifier, balanced immune functions is common and youthfulness (Ogbonnia *et al*., 2011). However, in most cases, where these herbal medicines are employed, their utilization is always based on long-term administration (Okada *et al*., 2006).

From our ethnomedicinal finding, Febi super bitters is taken 12 or 24 h interval for the cleansing of the blood, detoxifier, regulating blood sugar and body weight, general body pains, keeping healthy etc. However, it was allegedly used over a long period of time without the consideration of chronic effects that could ensue. Consequently, this study was carried out to scientifically investigate the immunomodulatory effect of the repeated sub-chronic administration of Febi super bitters in male Wistar rats.

MATERIALS AND METHODS

Herbal bitters: Febi super bitters are products of Dominion Nigeria International, 2 Victory Street, Iba Tedo, Ojo, Lagos. Nigeria.

Blood glucose glucometer: Accu-chek active glucometer and test strips, products of Roche Diagnostic Gmbh, D-68298 Mannheim, Germany were used for the fasting blood glucose level determination.

Quantitative assay kits: The kits for the determination of rat Interleukin-2 (IL-2), Interleukin-6 (IL-6) and Tumour Necrosis Factor-alpha (TNF-α) were products of RayBiotech, Inc. USA, while those for Total Cholesterol, Triacylglyceride, High Density Lipoprotein Cholesterol (HDL-C) and Uric Acid were products of LABKIT, CHEMELEX, S.A. Pol. Canovelles-Barcelona, Spain. Reduced Glutathione assay kit was a product of BioAssay Systems, Hayward, USA.

Other reagents: All the chemicals and reagents used 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: Eighteen, 8-10 weeks old male Wistar rats 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).

Methods

Experimental animals and procedure: The eighteen male Wistar rats were randomly grouped into three, comprising of six rats per group with an average body weight of 86.2±4.43 g. 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 administration was commenced. Calculated doses of the herbal bitters in mL kg^{-1} body weight for human adults as contained in the manufacturer's pamphlet were administered to male Wistar rats with slight modifications as illustrated:

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

The feed intake of the rats were monitored daily and prior to the administration of the herbal bitters and every interval of 7 days, the fasting blood glucose levels and the body weights of the animals were recorded. 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 h and the blood glucose level and body weights determined. This study was conducted in accordance with the National

Institutes of Health's Guide for the Care and Use of Laboratory Animals (NIH, 1985).

Haematological analysis: The haematological parameters were analysed by the automated haemology analyzer (SYSMEX K2X1: SYSMEX CORPORATION, JAPAN).

IL-2, IL-6 and TNF-α determination: The serum level of IL-2, IL-6 and TNF-**α** was determined by *in vitro* enzyme linked immunosorbent assay (ELISA) kit, using colourimetric reaction method as instructed in the kit manual with cat#ELR-IL2-001, ELR-IL6-001 and ELR-TNF **α**-001, respectively.

Blood glucose determination: The blood glucose concentration was determined by glucose oxidase reaction, using Accu-chek active glucometer and test strips. Glucose oxidase chromogen indicators and non-reactive agents are contained in the reagent pad to which about 2 μL of whole blood was applied.

Serum lipid profile determination

Total cholesterol: The serum total cholesterol level was determined by cholesterol oxidase-peroxidase (CHOD-POD) enzymatic colourimetric reaction, according to the method as described by Naito (1984a).

Triglyceride (TAG): The serum triglyceride level was determined by glycerol oxidase-peroxidase (GPO-POD) enzymatic colourimetric reaction, according to the method as described by Fossati and Prencipe (1982).

High density lipoprotein cholesterol (HDL-C): The serum HDL-C cholesterol level was determined by precipitation and cholesterol oxidase-peroxidase (CHOD-POD) enzymatic colourimetric reaction, according to the method as described by Naito (1984b).

Low density lipoprotein cholesterol (LDL-C) and very low lipoprotein cholesterol (VLDL-C): The VLDL-C cholesterol and LDL-C were determined by computation, according to the methods described by (Friedewald *et al*., 1972).

Serum uric acid determination: The serum uric acid level was determined by uricase-POD enzymatic colorimetric reaction, according to the method described by Schultz (1984).

Liver reduced glutathione determination: The levels of reduced glutathione (GSH) were determined using 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB) enzymatic colorimetric reaction, according to the method described by Baker *et al*. (1990).

Liver malondialdehyde determination: The concentration of thiobarbituric acid reactive substances, malondialdehyde (MDA) was determined using the method by Fraga *et al*. (1988).

Statistical analysis: This research work was completely randomised design (CRD) and the results were expressed as mean of 5 replicates±standard error of mean (SEM). 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

The results were presented as mean of five determination±standard error of mean (SEM) and significantly different ($p<0.05$) mean values were denoted by different alphabets.

Rat behaviour and morphology: After 22 days of administration of Febi super bitters at 0.462 mL kg^{-1} body weight, the rats were lean with bulged eyes and were very aggressive when disturbed, but less active when left unattended to. At 0.308 mL^{-1} kg body weight of Febi super bitters, no obvious signs of toxicities were seen in the rats when compared to the control, but were less active when left unattended.

Feed intake, Body weight and blood glucose concentration: The results obtained in the feed intake, body weight and blood glucose concentrations are presented in Table 1, Fig. 1 and 2, respectively. Significant decreases ($p<0.05$) were recorded in the feed intake (a measure of appetite), body weight (Fig. 1) and blood glucose (Fig. 2) of rats administered with the herbal bitters (Table 1).

Serum IL-2, IL-6 and TNF-α, uric acid and lipid profile and liver reduced glutathione and malondialdehyde concentrations: The patterns obtained following the administration of the herbal bitters on serum interleukin-2 (IL-2), interleukin-6 (IL-6), tumour necrosis factor alpha (TNF-α), lipid profile, uric acid concentration and liver concentrations of reduced glutathione (GSH) and malondialdehyde (MDA) in male rats are presented in Table 2. The serum IL-2 concentrations were not affected significantly ($p>0.05$) at the different doses of the herbal

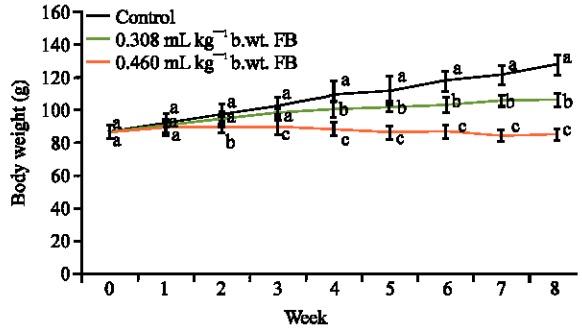

Fig. 1: Body weights of rats following the administration of the herbal bitters. Values are means±SEM; n = 5. Values bearing different alphabets are significantly different (p<0.05). FB (Febi super bitters)

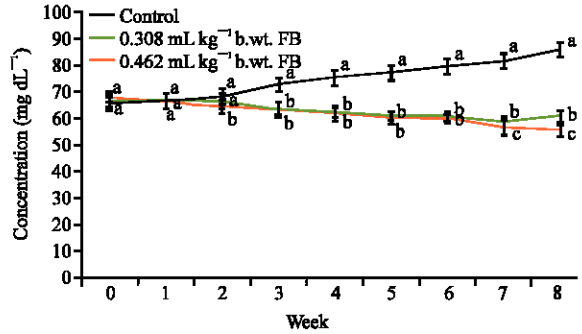

Fig. 2: The trends of the fasting blood glucose concentration in male rats. Values are means±SEM; n = 5. Values bearing different alphabets are significantly different (p<0.05). FB (Febi super bitters)

bitters, while significant increases were presented in the serum IL-6 and TNF-α concentrations (p<0.05). In Table 2, the administration of the Febi super bitters decreased significantly (p<0.05) the serum total cholesterol, triacylglycerides (TAG), low density lipoprotein cholesterol (LDL-C) concentrations and the atherogenic index (LDL-C/HDL-C), but did not affect significantly (p>0.05) the serum concentrations of high density lipoprotein cholesterol concentrations (HDL-C) and very low density lipoprotein cholesterol (VLDL-C). Significant increases (p<0.05) were obtained in the serum uric acid following the administration of the herbal bitters, while the concentrations of liver GSH and MDA decreased and increased respectively at 0.462 mL kg^{-1} body weight only of the herbal bitters (Table 2).

Haemeatological parameters: The effect of the repeated administration of the herbal bitters on the haematological parameters in male rats is depicted in Table 3. The Packed Cell Volume (PCV), Red Blood Cell (RBC) and haemoglobin (Hb) counts, Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC)

Table 1: Effect of sub-chronic administration of Febi super bitters on Feed intake

	Doses (BW)		
Week	Control	0.308 (FB)	0.462 (FB)
0	100.71±5.55^a	104.32±2.95^a	102.08±5.03^a
1	109.26±5.07^a	105.38±5.91^a	95.64±3.92^b
2	112.33±3.89^a	100.39±3.11^b	90.35±2.98^c
3	109.26±5.07^a	92.78±4.07^b	73.83±4.05^c
4	114.53±7.22^a	90.15±3.68^b	73.35±3.62^c
5	110.59±4.19^a	92.56±4.12^b	70.90±4.22^c
6	102.99±6.22^a	83.66±3.45^b	64.18±3.38^c
7	118.65±4.92^a	82.17±4.52^b	60.33±4.02^c
8	122.44±5.38^a	70.05±5.27^b	60.77±3.09^c

Values are means±SEM; n=5. Values bearing different alphabets are significantly different (p<0.05). g (gramme), BW (mL kg^{-1} body weight), FB (Febi super bitters)

Table 2: Serum cytokines, lipid profile, uric acid and liver reduced glutathione and malondialdehyde concentrations in rats administered with the herbal bitters

	Doses (BW)		
Parameter	Control	0.308 (FB)	0.462 (FB)
IL-2 (pg mL^{-1})	28.22±2.17^a	28.49±2.02^a	31.88±2.67^a
IL-6 (pg mL^{-1})	320.41±11.54^a	382.50±10.00^b	374.05±9.64^b
TNF-α (pg mL^{-1})	441.32±12.45^a	610.74±15.63^b	845.65±15.70^c
Total cholesterol (mg mL^{-1})	215.82±6.11^a	181.29±5.54^b	161.34±9.02^c
Triacylglyceride (mg mL^{-1})	205.62±4.72^a	189.45±8.12^b	172.88±6.64^c
HDL-C	45.21±2.19^a	44.15±3.31^a	44.65±4.04^a
VLDL-C	43.45±1.55^a	39.25±2.01^a	41.74±1.91^a
LDL-C	144.22±5.15^a	137.35±3.96^b	130.54±4.67^b
LDL-C/HDL-C (NA)	3.94±0.15^a	2.91±0.25^b	3.02±0.45^b
Uric acid	5.25±0.86^a	6.50±0.48^b	6.81±0.52^b
GSH (mg mL^{-1})	1.48±0.22^a	1.43±0.31^a	1.29±0.37^b
MDA (U)	5.19±0.55^a	5.23±0.82^a	5.85±0.74^b

Values are Means±SEM; n = 5. Values bearing different alphabets are significantly different (p<0.05). Key: BW (mL kg^{-1} body weight), FB (Febi super bitters), na (not applicable) and U (nmole/mg of protein)

Table 3: Haematological parameters in rats administered with the herbal bitters

	Doses (BW)		
Parameter	Control	0.308 (FB)	0.462 (FB)
PCV (%)	38.8±2.03^a	34.55±1.48^b	35.20±1.66^b
RBC (10^{12}) L^{-1}	6.46±0.49^a	5.56±0.39^b	5.05±0.44^b
Hb (g dL^{-1})	12.88±0.68^a	10.44±1.01^b	10.93±0.90^b
ESR (mm^3 h^{-1})	2.7±0.50^a	2.90±0.55^b	3.05±0.45^b
MCV (fL)10^{15}	52.55±2.85^a	44.45±2.05^b	43.35±1.96^b
MCH (10^{-12})	0.14±0.01^a	0.11±0.02^b	0.10±0.01^b
MCHC (g LC)	2.99±0.21^a	2.39±0.12^b	1.98±0.23^b
WBC 10^9 (L)	4.15±0.49^a	5.90±0.75^b	6.05±0.59^b
Lymphocyte (%)	32.4±4.22^a	36.50±2.68^b	37.70±3.05^b
Neutrophil (%)	52.9±4.01^a	49.30±3.17^a	51.40±2.89^a
Monocyte (%)	9.2±0.97^a	8.80±0.66^a	9.10±1.62^a
Eosinophil (%)	1.25±0.25^a	1.50±0.25^a	2.50±0.25^b
Basophil (%)	1.00±0.00^a	1.00±0.00^a	3.00±0.50^b
Platelets (μL^{-1})10^3	475.0±8.23^a	570.30±9.45^b	565.50±7.55^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)

were all significantly decreased ($p<0.05$) following the administration of the herbal bitters (Table 3). The Erythrocyte Sedimentation Rates (ESR), total White Blood Cell (WBC), Lymphocytes (L) and platelets counts in rats administered with the herbal bitters were increased significantly ($p<0.05$). Administration of the herbal bitters did not affect significantly ($p>0.05$) the neutrophil and monocyte counts, while the eosinophil and basophil counts were increased significantly ($p<0.05$).

DISCUSSION

In recent years, herbal bitters or phytomedicine have been commonly used in developing and developed countries in the modulation of immune response (Okada *et al*., 2006). Previous investigations have postulated that extracts of medicinal plants can provide supportive therapy to conventional chemotherapy.

The observed changes in the morphological appearance and physical activities of the rats administered with 0.462 mL kg^{-1} body weight of Febi super bitters could suggest that the herbal bitters was not well tolerated by the animals or potentially toxic at the dose. Although, none of the rats died during the length of this study, however, the recorded changes may make the herbal bitters to be categorized as potentially toxic at the dose of 0.462 mL kg^{-1} body weight (OECD, 2002). The reduction in the feed intake (Table 1) in the rats administered with the herbal bitters might be due to slow metabolism of ingested food substances in the gastrointestinal tract or the depression of appetite. Thus, the administration of Febi super bitters might have interfered with carbohydrate, protein or fat metabolism in the rats at the dose. Aniagu *et al*. (2005) reported that the loss of body weight or appetite in animals administered with chemical substances is an indication of the alteration of the nutritional "status' or 'benefits' of the animals.

The reductions obtained in the body weights of rats administered with Febi super bitters (Fig. 1) supported the result of the feed in take (Table 1), which is also in support of the Manufacturers claim. Although, most plant regimes that have been previously reported to possess immunomodulatory activities were shown to be involved in the down regulation / maintenance of body weight (Langley-Evans and Carrington, 2006; Oyewo *et al*., 2012). However, a critical examination of the trend in the body weight of rats administered with the herbal bitters at 0.462 mL kg^{-1} body weight (Fig. 1) suggested a chronic loss in body weight (catechia), as the rats showed no relative increase in body weight during the period of the experiment.

The administration of the herbal bitters did not stimulate/sensitize the immune system in the rats to up regulate/down regulate the serum concentration of IL-2 as depicted in Table 2. The trend obtained in the serum IL-6 concentration in rats administered Febi super bitters at 0.308 mL kg^{-1} body weight supported the immunomodulatory activities, while at 0.462 mL kg^{-1} body weight might indicate systemic inflammatory process (Table 3). This is because IL-6 can act as both a pro-inflammatory and anti-inflammatory cytokine, depending on the serum concentration (Oyewo *et al*., 2012). As pro-inflammatory cytokine, IL-6 protects against tissue damage by increasing the synthesis of fibrinogen as part of healing processes (Murtaugh *et al*., 1996) and also as an anti-inflammatory cytokine through its inhibitory effects on TNF-α (Heinrich *et al*., 1990). Reduced serum IL-6 levels are not of clinical diagnostic importance, except at very low concentration. However, over-expression of IL-6 has been reported in some disease conditions such as atherosclerosis due to increased fibrinogen production, sepsis, liver diseases, degenerative disease (cancer), oedema, massive weight loss and inflammatory disorders (Smolen and Maini, 2006; Dubinski and Zdrojewicz, 2007).

The increased the serum TNF-α concentrations in the herbal bitters administered rats (Table 3) indicated the induction of inflammatory processes, especially at 0.462 mL kg^{-1} body weight of the herbal bitters. The primary role of TNF-α is in the regulation of immune cells by regulating the growth of normal cells, inducing apoptotic cell death, inducing systemic inflammation and the inhibition of tumourigenesis and viral replication (Locksley *et al*., 2001). On the contrary, high serum levels of TNF-α has been implicated in increased risk of mortality, heart disease, septic shock, dehydration, anorexia, net catabolism, weight loss, anaemia, hepatosplenomegaly, autoimmune disorders and increased risk of cancer (Cesari *et al*., 2003; Dubinski and Zdrojewicz, 2007; Oyewo *et al*., 2012). The pattern in the serum TNF-α concentration supported the induction of systemic or localized inflammatory responses in male rats administered Febi super bitters, which could be responsible for the drop in feed intake (Table 1), body weight (Fig. 1), the lean structure at 0.462 mL kg^{-1} body weight and serum IL-6 (Table 2). Since IL-6 and TNF-α were implicated now as better predictors of inflammatory related problems in people with no conventional risk factors (Morrow and Ridker, 2000). Therefore, the down-regulation of the release of these cytokines is very important to enhance the activities of the immune system and reduce the possibility of developing degenerative diseases (Oyewo and Akanji, 2011).

The results of the erythrocyte parameters (PCV, RBC, Hb, MCV, MCH, MCHC and ESR) suggested that the administration of the herbal bitters caused anaemia in rats

(Table 3). The trend in (MCV) indicated that the sizes of RBCs were reduced, implicating microcytic anaemia, which is probably due to iron deficiency and chronic inflammatory response. In addition, the Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) supported the possibilities of iron deficiency anaemia and/microcytic hypochromic anaemia (haemoglobinization is incomplete with central parlour more than 8 μm) (Topley, 1998). The white blood cell count and lymphocytes in rats administered the herbal bitters (Table 3) suggest that the herbal bitters stimulated the immune cells, thereby supporting the induction inflammatory responses in the rats. Leukocytes are involved in fighting infection and clearing off injured or dead cells and tissues in body (Berg *et al*., 2001).

The increase in the platelets counts in rats following the administration of Febi super (Table 3) is not in agreement with the anaemic capability of the herbal bitters. However, the result may indicate an increased level of tissue healing processes or systemic inflammatory responses. That is, macrophages and neutrophils stimulate the increase in the production of blood platelets via increased biosynthesis of adenosine diphosphate (ADP) and the inhibition of Nitric Oxide (NO) production, triggered by tissue injury or damage (Ford and Giles, 2000). Therefore, the result of the blood platelets counts supported the trends obtained in the serum IL-6 and TNF-α concentrations (Table 2), as increased serum IL-6 and TNF-α concentrations are reported to induce the release acute phase proteins such as fibrinogen in blood.

The trends obtained in the blood glucose levels in male rats administered with Febi super bitters (Fig. 2) indicated that the herbal bitters interfered with the absorption and utilization of glucose. Although, the Manufacturer alleged that Febi super bitters ought to regulate the blood glucose level. However, the pattern obtained in our study depicted hypoglycaemic state, as the concentrations of blood glucose were later reduced below the reference points (as stated in the Accuchek glucometer manual; 65 mg dL^{-1}). The hypoglycaemic state might not be desirable, even when immunomodulatory regimes are known to possess blood glucose reducing capabilities (Digirolamo, 1994; Langley-Evans and Carrington, 2006; Oyewo *et al*., 2012). This is because long term hypoglycaemic conditions have been implicated in life threatening clinical conditions, such as weakness, oedema, ions imbalance, ketosis and neuoglycopenia that results from brain damage, caused by prolonged inadequate supply of glucose to the central nervous system (Balch and Balch, 1997; Berg *et al*., 2001).

The result of the serum total cholesterol, triacylglycerol and low density lipoprotein cholesterol in male rats administered the herbal bitters (Table 2) supported the manufacturer's claim that Febi super bitters helps to reduce the concentration of blood lipid. The probable mechanism of the reduction in blood lipid levels might be through the inhibition of the absorption of dietary lipid in the small intestine (Evers, 2008) and the inhibition of cholesterol biosynthesis in the liver (Sinclair *et al*., 2001). The increase in the serum uric acid concentration following the administration of Febi super bitters in rats (Table 2) could indicate oxidative stress or localized inflammatory responses. Uric acid is a more potent reducing agent in vivo than ascorbic acid and contributes more than a half of the total antioxidant capacity of blood plasma in mammals (Baillie *et al*., 2007). However, increased serum uric acid levels are reported in cases involving trauma or oxidative stress or inflammatory disorders (Xiang *et al*., 2001; Short *et al*., 2005).

The result obtained in the reduced glutathione (GSH) concentration in the liver in rats administered 0.462 mL kg^{-1} body weight of the herbal bitters (Table 2) supported the result obtained in the serum uric acid concentrations. This is because the plasma uric acid concentrations are tightly regulated so as to augment any drop in the concentration of reduced glutathione in the liver, mostly in oxidative stress conditions (Xiang *et al*., 2001). The trends obtained in the liver malondialdehyde (MDA) concentrations (Table 2) supported the result of the liver reduced glutathione and uric acid concentrations. Thus the administration of Febi super bitters at 0.462 mL kg^{-1} body weight induced oxidative stress in rats, which is a major factor that compromises the immune system through the induction of inflammatory responses by free radicals.

CONCLUSION

The sub-chronic administration of the Febi super bitters, even below the recommended dose did not present the alleged immunomodulatory potentials, but rather triggered inflammatory responses in male rats. Therefore, the daily use of the herbal bitters is not recommended.

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