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# Safety Evaluation of Two Nigerian Polyherbal Formulations (Fidson Bitter® And Daily Living Bitter®) In Male Wistar Rats

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## Abstract

**Background:** Herbal bitters are used for diverse diseases based on the manufacturers' assertions. However, little is known about their toxicity profile.

**Objective:** The safety profile of two commonly used herbal bitters in Nigeria (Fidson bitter® and Daily living bitter®)was evaluated in rats.

**Materials and Methods: S**ingle oral dose, 2 g/kg, of each reconstituted bitter extract was administered to male and female rats in acute toxicity test. Animals were observed for 14 days for behavioral changes and mortality. In sub-acute oral toxicity experiment, 250 mg/kg and 500 mg/kg of each bitter was separately administered daily to different groups of male Wistar rats for 30 days. Safety profile of concurrent administration of these bitters was also assessed. Histopathological, hematological, and clinical chemistry indices were evaluated.

**Results:** The $LD_{50}$ was found to be >2 g/kg. Daily living bitter® (DLB) had no significant effect on any of the indices evaluated ($P>0.05$). However, Fidson bitter® caused significant reductions in white blood cells count (WBC) compared with the control. Concomitant administration of the bitters resulted in significant ($P<0.05$) weight gain (up to 33 %), reduction in WBC and congestion of the liver without corresponding increase in liver biomarkers.

**Conclusion:** Daily living bitter® was safe in sub-acute administration while Fidson bitter®and combination of the two bitters reduced white blood cell count. Hence, caution should be exercised in using Fidson bitter® or combination of the two bitters in humans as findings suggest possibility of immune suppression.

**Keywords:** Toxicity profile, Polyherbal, Herbal bitters, Hematology, Wistar rats.

## INTRODUCTION

In the early 1960's,the advent of improved scientific methods for drug synthesis and manufacturing gradually encouraged the use of conventional medicines more than the then popular herbal medicines (Pan *et al*. 2014). By the 1980's reports of iatrogenic effects related to conventional medicines began to emerge and gradually a re-awakening of the relative safety of herbal products resurfaced with increasing use worldwide (Ekor, 2014). World Health Organisation (WHO) in 1974 encouraged the use of herbal medicines to "fulfil a need unmet by modern medicines" in the developing countries (Winslow and Kroll, 1998). Later WHO estimated that up to 80% of the world's population rely on traditional medicinal system for some aspect of primary health care (Farnsworth *et al*. 1985).

In regions where traditional medicines are frequently used in health care practices herbs are not often used for acute conditions, unless conventional medicines are out of reach, expensive or inconvenient, or because of their adverse effects (Wachtel-Galor and

Benzie, 2011). It is believed that conventional medicines offer no cure for chronic diseases, but a lifelong management (Wachtel-Galor and Benzie, 2011). Thus, switching to herbal medicine use in chronic disease gives the user a sense of taking action and being in control of their health (Thorne *et al*. 2002). Generally, herbal medicines are perceived to be healthier and safer than conventional medicines and their use is encouraged by religious and cultural beliefs (Jennings *et al*. 2014).

In most countries, especially the developing nations, there is a lack of quality control of these herbal products, such that the active ingredients and dosages are not standardized (Liang *et al*. 2004). Traditional healers who use these unstandardized herbal products, claim that polyherbal formulations (combination of different herbs) have synergistic or buffering effect, improves efficacy and ameliorate side effects (HemaIswarya and Doble, 2006; Aziz *et al*. 2013). This is in contrast with conventional medicines where polypharmacy is generally avoided.

Herbal medicines contain complex chemicals that are bioactive, thus, they are assumed to have multiple potential targets and mechanism of action, and are more efficacious than multiple conventional medicines for the same chronic disease(Attele *et al*. 1999; HemaIswarya and Doble, 2006). Contrary to the aforementioned, reported increase in morbidity and mortality with herbal medicines use have now become a source of concern globally and has drawn attention to its use (Bandarayanake, 2006).

Herbal bitters are examples of polyherbal formulations with increasing popularity globally as a result of unhindered direct to customer advertisement (Showande and Amokeodo, 2014). Our recent study reported the concomitant use of herbal bitters with conventional medicines and the concurrent use of two or more bitters. Participants in the study claimed that the concurrent use of herbal bitters hastens recovery from chronic diseases and speeds up weight loss (Showande and Amokeodo, 2014). Few herbal bitters' safety profiles have been documented(Aniagu *et al*. 2005; Akande *et al*. 2010; Ogbonnia *et al*. 2010). Some were reported to have adverse effect on the kidney and other organs of the body. These bitters included Bolex bitters®, Remedia mixture®, and Leon bitters®(Akande *et al*. 2010; Ogbonnia *et al*. 2010). Nature cure bitters® showed no adverse toxic effects in rats (Aniagu *et al*. 2005) while Bakers Cleansers® was hepatoprotective (Patrick-Iwuanyanwu *et al*. 2012).

There have been reported cases of adverse drug reactions and toxicity associated with herbal medicines especially polyherbal formulations (Gardner and McGuffin, 2013) and the use of more than one polyherbal formulations may further increase the incidence of these adverse drug reactions and toxicity. Fidson bitter®(FB) and Daily living bitter®(DLB) are among the popular herbal bitters frequently used in Nigeria(Showande and Amokeodo, 2014).The long term safety profiles of FB and DLB polyherbals when administered singly or concomitantly are yet to be reported. To this end, the safety profiles of these two polyherbal formulations were evaluated when administered individually and concomitantly on hematological, biochemical and histopathological indices in experimental animals.

## METHODOLOGY

### Materials and reagents

*Chemicals and reagents*

Daily living bitters and Fidson bitters were purchased from a local registered pharmacy in the city of Ibadan, Oyo state, Nigeria. Daily living bitters (DLB) produced by Sagopha Laboratories India and marketed by Neros Nigeria Limited (Batch number 08111-XK, National Agency for Food and Drug Administration and Control, NAFDAC, number A7-0124L). Fidson bitters (FB) produced by V.S International PVT LTD India, and marketed by Fidson Healthcare PLC Nigeria (Batch number VG1401, NAFDAC number A7-1018L). A 200 mL DLB contain aqueous extract from *Cynara scolymus* (11% w/v), *Smilax globra* (11% w/v), *Orthosiphon spiralis* (5.5% w/v g), *Cassia tora* (11% w/v), *Lactucaindica* (5.5% w/v), *Polygonum aviculare*(11% w/v),and *Passiflora foetida* (5.5% w/v). Each 100 mL of FB contain aqueous extract derived from *Aloe vera* (6% w/v), *Phyllanthus annun* (5.2% w/v), *Eclipta alba* (4% w/v), *Cassia augustifolia* (2.7% w/v), *Tephrosia purpurea* (2.4% w/v), *Cinnamomum zeylanicum* (1% w/v), *Citrus reticulate* (1% w/v), *Swertiachirata* (0.225% w/v), and Ginseng (0.02% w/v).

Assay kits for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Blood Urea Nitrogen (BUN), Serum Creatinine (SCr), Total Bilirubin (TBIL), and Total cholesterol (TCOL) were purchased from Randox Laboratories, Crumlin, Northern Ireland, United Kingdom. All other chemicals and reagents used were of analytical grade.

### Preparation of herbal bitter concentrates

Two liters each of DLB and FB with the same batch numbers, (08111-XK) and (VG001) respectively, were concentrated with a rotary evaporator at $40^0$C (Buchi Rotavapor R-210, model number:

0800014803, Switzerland) and freeze dried with a Lyotrap plus® freeze drier (model number: 912350, Great Britain) to determine the average weight of concentrate in each bottle of bitters. The freeze-dried extracts were stored in the fridge until ready for use.

**Phytochemical screening**

Total alkaloids, saponins, and flavonoid content of each herbal bitter concentrate were determined by the method described by Krishnaiah *et al*. (2009).

**Animals and housing conditions**

A total of sixty-five Wistar Wistar rats (6 - 8 weeks old) weighing 130-230 g (10 females and 55 males) used in the study were purchased from the Animal House of College of Medicine, University of Ibadan, Oyo state, Nigeria. They were kept in a well-ventilated polyethylene cage with five rats in each cage. The rats were fed with standard pellet and were allowed access to water *ad libitum*. They were acclimatized for two weeks and maintained under standard laboratory conditions.

**Toxicity studies**

**Acute oral toxicity study**

In the acute oral toxicity test, four groups of five rats each were used. Group A1 and A2 represented Fidson bitter group and had five male rats and five female rats, respectively. Likewise, the Daily living bitter group (Group B1 and B2) had similar numbers of male and female rats in separate cages. Group A1 and A2 received single oral dose of 2 g/kg body weight of reconstituted FB extract. Also Group B1 and B2 received same dose of reconstituted DLB extract according to a previously described method (Yamanaka *et al*. 1990). Prior to the administration of each herbal bitter, the animals were fasted overnight for 16 hand food withheld for a further 4 h post administration of herbal bitters. The rats were observed individually at least once during the first 30 min post-administration of the bitters, periodically during the first 24 h and daily thereafter for a period of 14 days. Signs of behavioral changes and number of death were recorded.

**Subacute oral toxicity study**

One-fourth (500 mg/kg) and one-eighth (250 mg/kg) of the maximum safe dose obtained from acute toxicity test were selected for sub-acute oral toxicity experiment. Forty-five male Wistar rats were randomly divided into nine groups of five rats each to evaluate the safety effect of administering the herbal bitters individually (Groups D to G) and concurrently (Groups H to K). The dose for Daily living bitters for human (an adult male) is two teaspoonful three times daily (30 mL per day) while the dose for Fidson bitter is two teaspoonful twice daily (20 mL per day). The two doses selected for this study in the animals were considered as low dose (250 mg/kg body weight) and high dose (500 mg/kg body weight) for each herbal bitter. The treatment groups were

Group C: Control group – received only water (10 mL/kg)
Group D: Received low dose of DLB (250 mg/kg body weight)
Group E: Received high dose of DLB (500 mg/kg body weight)
Group F: Received low dose of FB (250 mg/kg body weight)
Group G: Received high dose of FB (500 mg/kg body weight)
Group H: Received low dose of DLB (250 mg/kg body weight) + low dose of FB (250 mg/kg body weight)
Group I: Received high dose of DLB (500 mg/kg body weight) + high dose of FB (500 mg/kg body weight)
Group J: Received low dose of DLB (250 mg/kg body weight) + high dose of FB (500 mg/kg body weight)
Group K: Received high dose of DLB (500 mg/kg body weight) + Low dose of FB (250 mg/kg body weight)

The above treatments were administered to each rat by oral gavage once daily in the morning for 30 days. During this period the animals were observed for any behavioral and physical evidence of adverse effects or death. The experiment was performed in accordance with American Psychology Association guidelines (American Psychological Association, 1986).

**Parameters evaluated**

*Food consumption*

The amount of feeds consumed was measured daily from the quantity of feed supplied and the amount remaining after 24 h. Each group of animals was supplied with 150 g of rat feed each morning.

**Body weight and relative organ weight**

Each animal weight was also measured every 5 days. On the 30$^{th}$day of the experiment, the animals were euthanized with diethyl ether and five organs (lungs, heart, kidneys, liver, and spleen) were excised, adhering tissues removed, rinsed with normal saline and weighed immediately. Paired organs were weighed separately. Relative organ weight (100 x organ weight/body weight) was calculated for each organ.

**Hematology and clinical chemistry analyses**

Three milliliter blood samples were collected on the 30$^{th}$day of the experiment from the retro-orbital sinus of the rats under light chloroform anesthesia. and shared into EDTA bottles and lithium heparinized bottles. These were used for the determination of hematocrit (HCT), hemoglobin (HB), red blood cells count (RBC), white blood cells count (WBC), platelets (PLAT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and differential leukocyte counts [neutrophils (NEU), monocytes (MON), lymphocytes (LYM) and eosinophil (EOS)]. These parameters were determined by methods described in Davies *et al* (1984).

To determine the levels of ALT, AST, TBIL, BUN, SCr, TCOL and total protein (TPRO), blood samples in the heparinized tubes were immediately centrifuged at 4000 *g* for 10 min and the plasma analysed with Randox kits.

**Microscopic pathology**

After the excision of the organs, the tissues were fixed in 10% formalin and processed in automatic tissue processor. Tissues were then embedded in paraffin wax using Embedding system (Leica EG 1160). They were thereafter sectioned with a microtome at 4 microns slide. The slides were fixed on hot plate for about 30 min and sections stained with Hematoxylin and Eosin (Avwioro, 2011). Histopathological assessment and photomicrography of the prepared slides were done by a pathologist, using an Olympus Light Microscope attached with Kodak digital camera.

**Ethical approval**

The ethical approval for the study was obtained from the University of Ibadan/University College Hospital Ethics Review Committee. The ethical approval number was UI/EC/13/021.

**Statistical analysis**

Data were expressed as mean ± standard error of the mean (S.E.M). Analysis of variance (ANOVA) was performed to determine the differences in means among the treatment groups and Dunnette-t and Games Howell post hoc tests were used based on the level of significance of Levene statistics (homogeneity of variance). Statistical analyses were performed with Statistical Package for Social Science (SPSS) version 20 and level of significance was set at $P<0.05$.

**RESULTS**

The concentration of extracts in each bottle of Daily living bitter (DLB) and Fidson bitter (FB) were 81.0 mg/mL and 91.5 mg/mL, respectively.

**Phytochemical content of bitters**

The percentage composition of saponins, flavonoids, and alkaloids in Fidson bitters was 2.93%, 71.97%, and 0.92% respectively; while Daily Living bitters had 4.35% saponins, 44.03% flavonoids, and 0.76% alkaloids.

**Acute oral toxicity study**

No visible evidence of toxicity was observed in both male and female rats administered with 2 g/kg body weight of DLB and FB. Soft stool was noticed in 2/5 female rats and 1/5 male rat in FB group from day 3 through day 5. Normal weight gain was observed in all the test groups.

**Sub-acute oral toxicity study**

**Weight changes, relative organ weight and**

Administration of the two herbal bitters individually or concomitantly did not result in death of the animals during the period of experiment. When the bitters were administered individually at low and high doses, weight gain was not significantly different from the control group ($P>0.05$) as shown in Table 1. Co-administration of high dose of FB with low dose of DLB resulted in a marginal weight gain (1.19%) which was statistically significantly different from the control group, 9.98%, ($P<0.05$)as shown in Table 2. The concurrent administration of low doses of DLB and FB and high doses of DLB and FB resulted in appreciable weight gain, $P<0.05$, when compared with the control group (Table 2). It was also noticed that in the second week of the experiment there was a moderate drop in weight of rats that received different combinations of FB and DLB concurrently.

**Table 1: Changes in body weight and relative organ weight of rats administered with Daily living bitter® or Fidson bitter®**

| Relative organ weight | Control | Dose of Daily living bitter (mg/kg) | | Dose of Fidson bitter (mg/kg) | |
|---|---|---|---|---|---|
| | | 250 | 500 | 250 | 500 |
| Heart | 0.36 ± 0.03 | 0.37 ± 0.01 | 0.40 ± 0.03 | 0.38 ± 0.04 | 0.37 ± 0.02 |
| Lung | 0.87 ± 0.16 | 0.66 ± 0.02 | 0.89 ± 0.17 | 0.70 ± 0.06 | 0.77 ± 0.07 |
| Liver | 3.11 ± 0.02 | 3.48 ± 0.13 | 3.27 ± 0.26 | 3.59 ± 0.22 | 3.75 ± 0.21 |
| Kidney | 0.59 ± 0.02 | 0.58 ± 0.12 | 0.62 ± 0.01 | 0.65 ± 0.06 | 0.62 ± 0.02 |
| Spleen | 0.37 ± 0.03 | 0.41 ± 0.01 | 0.34 ± 0.04 | 0.43 ± 0.05 | 0.49 ± 0.04 |
| % Body weight gain | 9.98 ± 0.82 | 5.93 ± 2.00 | 8.74 ± 3.04 | 7.80 ± 3.49 | 5.89 ± 2.61 |

Values represent mean ± S.E.M. $^{a}P<0.05$ and $^{b}P<0.01$ were considered significant in comparison with the control group.

**Table 2: Changes in body weight and relative organ weight of rats administratered Daily living bitter® and Fidson bitter® concurrently**

| Relative organ weight | Control | Dose of Fidson bitter (F) + dose of Daily living bitter (D) administered in mg/kg body weight | | | |
|---|---|---|---|---|---|
| | | 250F + 250D | 250F + 500D | 500F + 250D | 500F + 500D |
| Heart | 0.36 ± 0.03 | 0.37 ± 0.02 | 0.33 ± 0.03 | 0.42 ± 0.02 | 0.33 ± 0.03 |
| Lung | 0.87 ± 0.16 | 0.79 ± 0.07 | 0.72 ± 0.06 | 0.89 ± 0.12 | 0.76 ± 0.04 |
| Liver | 3.11 ± 0.02 | 4.15 ± 0.21$^{b}$ | 3.91 ± 0.06$^{b}$ | 3.98 ± 0.05$^{b}$ | 3.65 ± 0.10$^{b}$ |
| Kidney | 0.59 ± 0.02 | 0.72 ± 0.06 | 0.58 ± 0.04 | 0.68 ± 0.01$^{a}$ | 0.65 ± 0.02 |
| Spleen | 0.37 ± 0.03 | 0.40 ± 0.04 | 0.37 ± 0.03 | 0.52 ± 0.03$^{a}$ | 0.43 ± 0.03 |
| % Body weight gain | 9.98 ± 0.82 | 33.62 ± 5.73$^{b}$ | 14.73 ± 2.85 | 1.19 ± 0.51$^{a}$ | 25.47 ± 1.56$^{b}$ |

Values represent mean ± S.E.M. $^{a}P<0.05$ and $^{b}P<0.01$ were considered significant in comparison with the control group.

Significant increases in relative liver weight were observed in all the treatment groups when FB and DLB were co-administered (Table 2). Groups of rats treated with either low or high dose of FB or DLB did not show any significant change in organ weights (Table 1).

Rats administered with 500 mg/kg FB consumed more food than the control group from the 18$^{th}$ day till the end of the experiment while rats that received FB and DLB concurrently in various combinations, consumed lesser amount of food from the 8$^{th}$ to 14$^{th}$ day in comparison with the control group. Other groups did not show appreciable changes in the level of food consumed.

**Hematology**

Fidson bitter administered at a dose of 250 mg/kg caused a significant reduction in WBC, PLT, MCV, MCH, and LYM, but a significant increase in NEU, was also observed in comparison with the control group (Table 3). Higher dose of FB (500 mg/kg) also resulted in a significant decrease in LYM, $P<0.05$, (Table 3). No significant changes were observed with the two doses of DLB used on any hematological parameters compared with the control (Table 3).

Fidson and DLB given at combined doses of 500 mg/kg FB + 250 mg/kg DLB and 500 mg/kg FB + 500 mg/kg DLB caused a statistically significant reduction in PCV and HB, respectively, $P<0.05$, compared with the control group (Table 4). Another statistically significant reduction was observed in the values of WBC, and PLT when the four differently combined doses of FB + DLB were administered to the rats (Table 4). Other changes in hematological parameters are shown in Table 4.

**Table 3: Changes in hematological indices of rats administered with Daily living bitter® or Fidson bitter®**

| Indices | Control | Dose of Daily living bitter (mg/kg) | | Dose of Fidson bitter (mg/kg) | |
|---|---|---|---|---|---|
| | | 250 | 500 | 250 | 500 |
| HCT (%) | 52.20 ± 2.04 | 46.80 ± 1.39 | 48.60 ± 2.25 | 50.60 ± 2.25 | 47.00 ± 1.41 |
| HB (g/dL) | 17.33 ± 0.66 | 15.45 ± 0.56 | 16.30 ± 0.75 | 16.04 ± 0.83 | 15.56 ± 0.61 |
| RBC ($10^6$/µL) | 8.30 ± 0.21 | 7.73 ± 0.23 | 8.00 ± 0.30 | 8.58 ± 0.29 | 7.73 ± 0.30 |
| WBC ($10^3$/µL) | 8.59 ± 0.99 | 7.60 ± 1.02 | 6.57 ± 1.37 | 4.80 ± 0.59[a] | 6.05 ± 0.27 |
| PLAT ($10^5$/µL) | 1.48 ± 0.14 | 1.22 ± 0.13 | 1.03 ± 0.19 | 0.90 ± 0.06 [a] | 1.08 ± 0.05 |
| MCV (fl) | 62.80 ± 1.20 | 60.60 ± 0.40 | 60.40 ± 0.93 | 58.40 ± 1.21[a] | 60.40 ± 1.03 |
| MCHC (g/dL) | 33.40 ± 0.25 | 33.00 ± 0.32 | 33.60 ± 0.25 | 32.20 ± 0.56 | 33.20 ± 0.66 |
| MCH (pg) | 20.86 ± 0.46 | 19.99 ± 0.24 | 20.35 ± 0.29 | 19.35 ± 0.41[a] | 20.16 ± 0.49 |
| MON (%) | 2.00 ± 0.32 | 2.20 ± 0.20 | 3.40 ± 0.68 | 1.80 ± 0.37 | 1.80 ± 0.20 |
| LYM (%) | 78.60 ± 1.97 | 65.20 ± 10.98 | 76.00 ± 1.34 | 66.20 ± 1.28[b] | 65.40 ± 2.42[a] |
| NEU (%) | 17.80 ± 2.31 | 31.20 ± 9.74 | 19.20 ± 1.74 | 29.20 ± 1.24[a] | 29.20 ± 2.48 |
| EOS (%) | 1.60 ± 0.25 | 3.20 ± 1.24 | 1.40 ± 0.40 | 2.60 ± 0.40 | 3.60 ± 0.25 |
| TPRO (g/dL) | 7.70 ± 0.39 | 7.96 ± 0.10 | 7.00 ± 0.37 | 7.88 ± 0.21 | 7.70 ± 0.05 |

Values represent mean ± S.E.M. [a]$P<0.05$ and [b]$P<0.01$ were considered significant in comparison with the control group. Hematocrit (HCT), hemoglobin (HB), red blood cells count (RBC), white blood cell count (WBC), platelets (PLAT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), neutrophils (NEU), monocytes (MON), lymphocytes (LYM) and eosinophil (EOS), total protein (TPRO).

**Table 4: Changes in hematological indices of rats administratered with Daily living bitter® and Fidson bitter® concurrently**

| Indices | Control | Dose of Fidson bitter (F) + dose of Daily living bitter (D) administered in mg/kg body weight | | | |
|---|---|---|---|---|---|
| | | 250F + 250D | 250F + 500D | 500F + 250D | 500F + 500D |
| HCT (%) | 52.20 ± 2.04 | 45.60 ± 1.08 | 52.00 ± 0.84 | 40.00 ± 5.76[a] | 41.20 ± 1.99 |
| HB (g/dL) | 17.33 ± 0.66 | 15.07 ± 0.35 | 17.57 ± 0.34 | 13.07 ± 2.13 | 13.43 ± 0.61[a] |
| RBC ($10^6$/µL) | 8.30 ± 0.21 | 7.73 ± 0.14 | 8.48 ± 0.02 | 6.91 ± 1.04 | 6.90 ± 0.35 |
| WBC ($10^3$/µL) | 8.59 ± 0.99 | 5.22 ± 0.58[a] | 4.27 ± 0.30[b] | 5.42 ± 0.06[a] | 4.78 ± 1.13[b] |
| PLAT ($10^5$/µL) | 1.48 ± 0.14 | 0.96 ± 0.06[b] | 1.11 ± 0.06[a] | 0.95 ± 0.02[b] | 0.92 ± 0.12[b] |
| MCV (fl) | 62.80 ± 1.20 | 58.00 ± 0.45[b] | 61.20 ± 1.11 | 58.20 ± 0.86[b] | 59.60 ± 0.75 |
| MCHC (g/dL) | 33.40 ± 0.25 | 32.60 ± 0.25 | 33.80 ± 0.20 | 31.20 ± 0.97 | 32.00 ± 0.45 |
| MCH (pg) | 20.86 ± 0.46 | 19.48 ± 0.12[a] | 20.71 ± 0.42 | 18.57 ± 0.36[b] | 19.51 ± 0.37 |
| MON (%) | 2.00 ± 0.32 | 2.40 ± 0.40 | 3.00 ± 1.14 | 3.00 ± 0.45 | 2.60 ± 0.25 |
| LYM (%) | 78.60 ± 1.97 | 66.80 ±2.89 | 71.00 ± 1.96 | 72.00 ± 3.44 | 66.60 ± 2.25[a] |
| NEU (%) | 17.80 ± 2.31 | 28. 20 ± 2.35 | 23.00 ± 1.45 | 21.60 ± 3.42 | 28.00 ± 2.24 |
| EOS (%) | 1.60 ± 0.25 | 2.60 ± 0.25 | 3.00 ± 0.63[a] | 3.20 ± 0.20[a] | 3.00 ± 0.31[a] |
| TPRO (g/dL) | 7.70 ± 0.39 | 6.52 ± 0.34 | 7.30 ± 0.17 | 7.53 ± 0.12 | 6.93 ± 0.11 |

Values represent mean ± S.E.M. [a]P<0.05 and [b]P<0.01 were considered significant in comparison with the control group. Hematocrit (HCT), hemoglobin (HB), red blood cells count (RBC), white blood cell count (WBC), platelets (PLAT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), neutrophils (NEU), monocytes (MON), lymphocytes (LYM) and eosinophil (EOS), total protein (TPRO).

**Clinical chemistry**

Fidson bitter administered at a dose of 250 mg/kg reduced the level of ALT significantly in comparison with the control group (Table 5) while a significant increase in SCr level was observed in the groups of rats treated with combined low doses of FB and DLB, P<0.05, (Tables 6).

**Histology**

No visible lesions were observed in the kidneys, lungs, spleen, and heart of both the control and the treatment groups. Moderate portal congestion was observed in the groups treated with 250 mg/kg DLB (1 in 5 rats), 500 mg/kg FB (1 in 5 rats), and 250 mg/kg FB + 250 mg/kg DLB (4 in 5 rats). Severe diffuse vacuolar degeneration and necrosis of hepatocytes were seen in the group administered with 500 mg/kg FB (2 in 5 rats) while severe periportal cellular infiltration by mononuclear cells were observed in 250 mg/kg FB + 500 mg/kg DLB (5 in 5 rats) and 250 mg/kg FB + 250 mg/kg DLB (4 in 5 rats) treatment groups (Figure 1).

**Table 5: Changes in clinical chemistry indices of rats on individual and concurrent administration of Daily living bitter® and Fidson bitter®**

| Indices | Control | Dose of Daily living bitter (mg/kg) | | Dose of Fidson bitter (mg/kg) | |
|---|---|---|---|---|---|
| | | 250 | 500 | 250 | 500 |
| AST (U/L) | 44.60 ± 0.87 | 47.00 ± 0.44 | 44.00 ± 1.05 | 43.00 ± 1.30 | 47.20 ± 1.07 |
| ALT (U/L) | 31.00 ± 0.95 | 31.60 ± 0.51 | 30.00 ± 0.89 | 27.60 ± 0.75* | 30.80 ± 1.02 |
| AST/ALT | 1.44 ± 0.03 | 1.49 ± 0.01 | 1.47 ± 0.05 | 1.56 ± 0.04 | 1.54 ± 0.05 |
| BUN (mg/dL) | 15.80 ± 0.80 | 17.60 ± 0.24 | 15.40 ± 0.68 | 14.80 ± 0.84 | 14.80 ± 0.92 |
| CRT (mg/dL) | 0.50 ± 0.06 | 0.76 ± 0.08 | 0.74 ± 0.12 | 0.62 ± 0.12 | 0.50 ± 0.10 |
| BIL (mg/dL) | 0.08 ± 0.01 | 0.18 ± 0.04 | 0.08 ± 0.01 | 0.12 ± 0.04 | 0.17 ± 0.04 |
| TCOL (mg/dL) | 47.80 ± 6.63 | 61.80 ± 8.57 | 67.40 ± 9.44 | 51.20 ± 5.84 | 57.60 ± 8.17 |

Values represent mean ± S.E.M. $^{a}P<0.05$ and $^{b}P<0.01$ were considered significant in comparison with the control group. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Blood Urea Nitrogen (BUN), Creatinine (SCr), Bilirubin (BIL), and Total cholesterol (TCOL).

**Table 6: Changes in clinical chemistry indices of rats on individual and concurrent administration of Daily living bitter® and Fidson bitter®**

| Indices | Control | Dose of Fidson bitter (F) + dose of Daily living bitter (D) administered in mg/kg body weight | | | |
|---|---|---|---|---|---|
| | | 250F + 250D | 250F + 500D | 500F + 250D | 500F + 500D |
| AST (U/L) | 44.60 ± 0.87 | 46.20 ± 0.37 | 46.20 ± 0.49 | 44.20 ± 1.16 | 43.00 ± 1.10 |
| ALT (U/L) | 31.00 ± 0.95 | 31.50 ± 0.29 | 32.60 ± 0.81 | 30.20 ± 1.02 | 29.80 ± 1.07 |
| AST/ALT | 1.44 ± 0.03 | 1.46 ± 0.02 | 1.42 ± 0.04 | 1.46 ± 0.03 | 1.45 ± 0.06 |
| BUN (mg/dL) | 15.80 ± 0.80 | 16.60 ± 0.25 | 15.60 ± 0.25 | 14.00 ± 0.45 | 14 ± 0.32 |
| CRT (mg/dL) | 0.50 ± 0.06 | 0.76 ± 0.75$^{a}$ | 0.60 ± 0.60 | 0.50 ± 0.06 | 0.35 ± 0.05 |
| BIL (mg/dL) | 0.08 ± 0.01 | 0.11 ± 0.02 | 0.08 ± 0.01 | 0.15 ± 0.04 | 0.11 ± 0.03 |
| TCOL (mg/dL) | 47.80 ± 6.63 | 68.00 ± 8.25 | 48.40 ± 3.30 | 56.20 ± 9.23 | 46.20 ± 2.89 |

Values represent mean ± S.E.M. $^{a}P<0.05$ and $^{b}P<0.01$ were considered significant in comparison with the control group. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Blood Urea Nitrogen (BUN), Creatinine (SCr), Bilirubin (BIL), and Total cholesterol (TCOL).

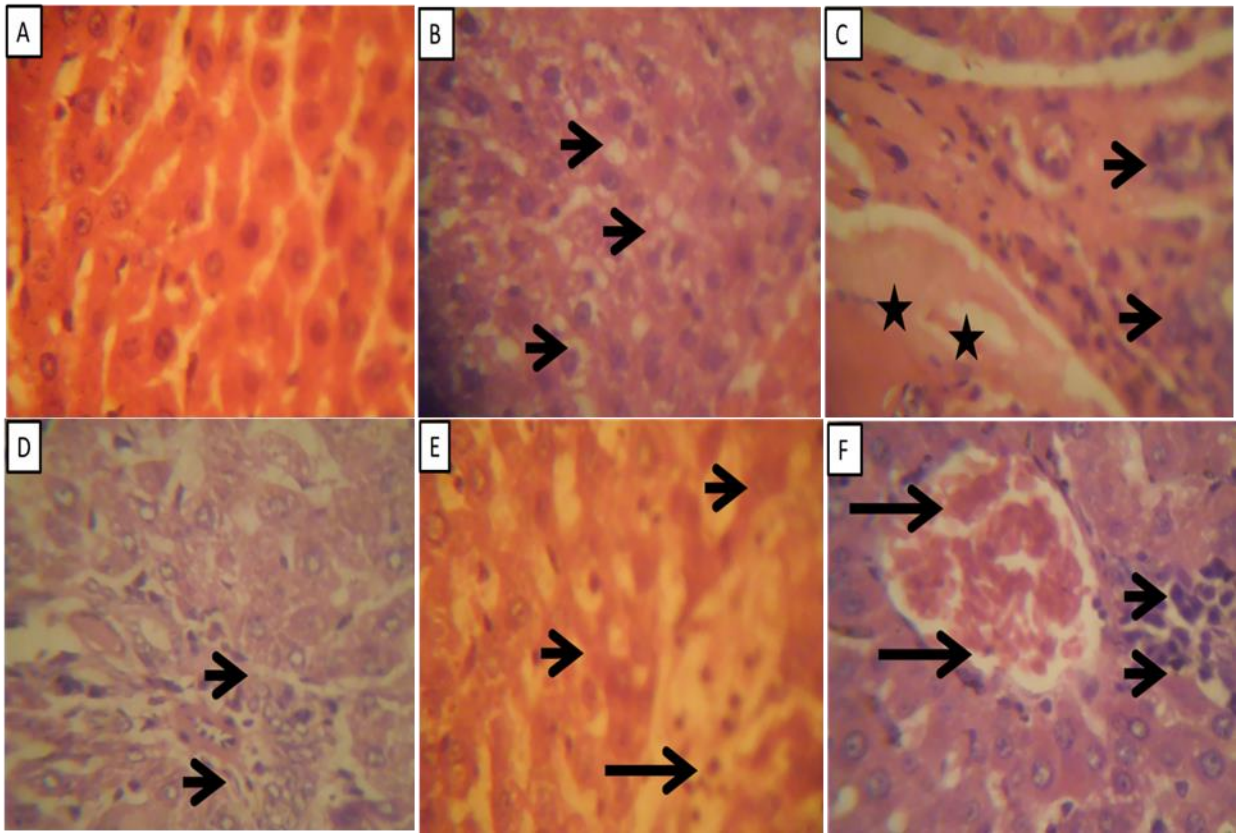


**Figure 1: Photomicrographs of liver sections, H & E stain, 400X magnification. (A) Liver of control group showing normal histology. (B) Liver of rats treated with 250 mg/kg DLB, showing moderate diffuse vacuolar degeneration of hepatocytes (arrows). (C) Liver of rats treated with 250 mg/kg FB + 500 mg/kg DLB, showing severe portal congestion, and edema (stars), moderate periportal cellular infiltration (arrows). (D) Liver of rats treated with 500 mg/kg FB, showing severe diffuse vacuolar degeneration and necrosis of hepatocytes, moderate periportal cellular infiltration by mononuclear cells (arrows). (E) Liver of rats treated with 500 mg/kg FB + 250 mg/kg DLB; the sinusoids are prominent as the hepatocytes appear shrunken (short arrows). There is moderate periportal cellular infiltration by mononuclear cells (long arrow). (F) Liver of rats treated with 250 mg/kg FB + 250 mg/kg DLB, showing mild portal congestion (long arrows) with severe periportal cellular infiltration by mononuclear cells (short arrows).**

## DISCUSSION

The toxic effect of herbal medicines is becoming rampant, especially when they are used for chronic diseases. There have been reported cases of hepatotoxicity, nephrotoxicity, blood, cardiovascular and nervous system toxicities with some herbs (Maffè *et al*. 2013; Teschke *et al*. 2013). These toxicities are common with single herbs but more frequent with the use of polyherbal formulations including herbal bitters (Maffè *et al*. 2013; Teschke *et al*. 2013). Despite the fact that, the use of polyherbal formulations is on the increase worldwide, few reports exist on their safety (Rosidah *et al*. 2009). Interestingly, there are many polyherbal formulations on the market in Nigeria, but scanty toxicity data are available for these products. We therefore investigated the effect of two polyherbal formulations widely used in Nigeria (Fidson bitter® and Daily living bitter®) on hematological, biochemical and histopathological indices in experimental animals, when administered singly and concomitantly.

In acute toxicity test, there was no sign of toxicity or death of the animals when the herbal bitters were administered at a single dose of 2 g/kg. This showed that the two herbal bitters have an approximate $LD_{50}$ greater than 4 g/kg (Yamanaka *et al*. 1990). Thus, they could be generally regarded as safe (GRAS) and of low toxicity (Clarke and Clarke, 1967).

Appreciable weight gain were recorded for rats treated with combined doses of FB and DLB in the sub-acute toxicity test in comparison with the control,. Increased appetite and the nutritive nature of the herbal bitters might have been responsible for the weight gain (Koithan and Niemeyer, 2010). It was,

however, noticed that before the weight gain there had been moderate weight loss in the second week which coincided with the reduction in food intake by these groups of animals from the 8th to 14th day of the experiment. This may be due to non-optimal utilization of food or suppression of appetite which resulted in initial temporary weight loss. This finding agreed with the claim of participants in a recent study, that the concomitant use of two herbal bitters hasten weight reduction (Showande and Amokeodo, 2014). Usually weight loss or weight gain in experimental animals is considered as a pointer to the adverse effect of drugs, chemicals or herbs (Mohamed *et al*. 2011; Witthawaskul *et al*. 2003). Excessive weight gain may be a source of concern if there was a corresponding increase in absolute and relative organ weights when the two herbal bitters were co-administered. In this study, a significant increase in the relative weight of the liver was observed. This was supported by histopathological findings which showed portal congestion and periportal cellular infiltration by mononuclear cell in more than 80 % of the rats treated with combined doses of FB and DLB. However, a corresponding increase in liver enzyme markers (ALT and AST) were not observed , perhaps due to the short duration of herbal bitters administration. Low dose of FB caused a significant reduction of ALT but in general, a reduction in ALT, is not a critical factor in toxicity study (Shin *et al*. 2012). Hence, this present sub-acute observed changes may not be toxicologically relevant.

Hematological indices in rats are highly predictive of risk of human toxicity, as a result of the administration of a toxic substance (Olson *et al*. 2000; Shanks *et al*. 2009). From our study, DLB did not exert any significant effect on hematological parameters, but low doses of FB had a significant impact on some of these indices. It resulted in reduction in WBC, PLT, MCV, MCH, and LYM. Reduction in WBC and LYM could be as a result of immunosuppression, and chronic suppression of lymphocytes may lead to reduced response to inflammation and infection (Haffor 2009). The significant reduction in HCT, HB, WBC, and PLT noticed when various dose combinations of the two herbal bitters were used was influenced by the dose of the bitters. Interestingly, increasing the dose of FB from 250 mg/kg to 500 mg/kg in these combinations lowered their values, but increasing the dose of DLB from 250 mg/kg to 500 mg/kg had no effect on these parameters. Fidson bitter seem to significantly affect the hemopoietic system when used alone or in combination. Fidson bitter contain about 70% flavonoids This group of phytochemicals has been reported to increase vascular integrity and they also act as antihemorrhagic but most importantly lower lipid peroxidation level which causes haemolysis of erythrocytes (Konaté *et al*., 2012; Mahmoud *et al*. 2012). This may explain their activities on hematological indices noticed in this study. However, it should be noted that despite these significant changes in the hematological values, they are still within normal range for Wistar rats of 6 - 8 weeks old except the reduction in WBC which is complete outside the normal range. Thus, FB or combinations of DLB and FB could result in immunosuppression.

The value of SCr was markedly increased when combined low doses of the bitters were administered to the rats. However, there were no matching histopathological changes to the kidneys of these rats. These changes in the biochemical markers for liver and kidney are also within the normal range, and thus cannot be regarded as adverse effects (Yam *et al*. 2009). Similar findings were reported for Bakers cleanser bitter and Leone bitter where a significant reduction in ALT and increase in SCr were respectively noticed (Ogbonnia *et al*. 2010; Patrick-Iwuanyanwu *et al*. 2012).

The total daily recommended dose of DLB and FB for a 70 kg man is 30 mL and 20 mL, respectively. Since the rat dose of 250 mg/kg and 500 mg/kg are approximately 7 – 14 and 9.5 – 19 times higher than the human daily dose for DLB and FB, respectively; a 70 kg man will then require a minimum daily dose of 190 mL of these bitters to elicit the hematological effect seen in this study. It is unlikely that an average human will take this volume of the bitters in a day, however caution should be exercised until human studies proved otherwise.

## Conclusion

Daily living bitter® was found to be relatively safe while Fidson bitter® and concomitant administration of the two bitters affected some vital hematological indices especially the white blood cell count (WBC) which may lead to immunosuppression. Hence, the two herbal bitters should not be coadministered and care should be taken when Fidson bitter® is used for a long period in humans until clinical data are available for informed decision.

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