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Original Article

# EFFECT OF BITTER EXTRA ON SELECTED HAEMATOLOGICAL PARAMETERS OF ADULT MALE WISTAR RATS

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## Abstract

**BACKGROUND:** Bitter Extra is a brand of herbal product adjudged to be efficacious in the treatment of various ailments.
**AIM:** This present study was aimed at investigating the effect of Bitter Extra on some hematological parameters of adult male Wistar Rats.
**METHODOLOGY:** Sixteen adult male Wistar Rats of weights ranging from 155g-230g were used and divided into four groups with four rats in each group. Group 1 served as the control and was given standard diet and water *ad libitum* while groups 2, 3 and 4 were taken as the test groups and administered with varying doses of Bitter Extra.
**RESULTS:** Among all the groups, there were no significant changes (P>0.05) in the mean body weights of the rats in the test groups compared with the control group which indicates that Bitter Extra has no effect on body weight. However, in the haematological parameters, the increased changes observed among the groups in Mean Cell Volume were significant (P<0.05). Also, the Mean Corpuscular Haemoglobin was significantly high (P<0.05) among all the groups when compared with the control group. The Mean Corpuscular Haemoglobin Concentration shows no significant difference in all the test groups when compared with the control group (P>0.05). At higher dose, Bitter Extra causes significant increase in White Blood Cell count, an indication of inflammatory property. Also, it causes insufficient production of RBCs at high dose resulting to iron deficiency hypochromic microcytic anemia.
**CONCLUSION:** These findings suggest that Bitter Extra is potentially harmful especially with repeated usage, necessitating the need to avoid indiscriminate use.

**Keywords:**
Bitter Extra, Haematological Parameters, Anaemia, Inflammation

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## Introduction

Plants (herbs or ethno botanicals) have been used from the beginning of human race and are still used throughout the world for promotion of health and treatment of diseases (Stojanoski, 1999). The World Health Organization (WHO) has defined herbal medicine as any part of the plant that can be used for therapeutic purposes or as precursors for the synthesis of important drugs (WHO, 2005). Based on the information from WHO, the use of herbal medicine worldwide has surpassed the use of conventional therapies by two to three times (Sanjoy and Yogeshwer, 2003). Plants and herbs form the basis of today's modern medicine and have contributed enormously to the commercial drug preparations manufactured today (Fabricant and Farnsworth, 2001). About 25% of the drugs prescribed throughout the world are manufactured from plants (Sanjoy and Yogeshwer, 2003). In most developing countries, herbs rather than conventional drugs are often used in health care services. For some individuals, herbal medicine is the preferred method of treatment, while for others; herbs are used as adjunct to therapy in conventional pharmaceuticals. However, in most developing countries of the world,



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traditional medicine of which herbal is a core part is the only system of health care that are accessible and affordable (Sissi andIris, 2011).

Herbal medicine has been reportedly used by about 80% of the world population both in the developing and developed countries where modern medicines are predominant (Rickert *et al.*, 1999; Ogbonna *et al.*, 2008). In Lagos State, South Western Nigeria, more than 60% of the surveyed population claimed to have used an herbal mixture either alone or in combination with other medicines (Ibrahim *et al.*, 2011). The rising popularity of phytomedicines could be attributed to the alleged advantages of being efficacious and also a more affordable source of medical care. In contrast, there is a growing disillusion with modern medicines coupled with the misconception that herbal supplements might be devoid of adverse and toxic effects, which are associated with conventional and allopathic medicines. But reports have raised concerns that indiscriminate use of packaged herbal bitters might have a toxic effect on the spleen, pancreas, heart and other organs in the body (Ezejiofor *et al.,* 2008).

Herbal supplements are administered in most clinical conditions over a long period of time, without taking cognizance of their toxic effects which might result from a prolonged usage (Park *et al.*, 2010). In most cases, these herbal products are not often prescribed by a physician neither were they dispensed by a pharmacist. The individual reports of any potential adverse effect of herbal bitters are mostly absent or inaccurate. Therefore, the dangers associated with the potential toxicity of many of these herbal products of which Bitter Extra is a brand and other herbal therapies, which are being used over long period of time demands that practitioners and even the general public be kept abreast of reported incidence of toxicities.

Owing to the growing incidences of many chronic diseases which affects some vital organs coupled with high consumption rate of bitter products it becomes imperative to carry out a study on the possible effects that may occur in blood parameters following Bitter Extra administration.

Bitter Extra Herbal Mixture is a 100% herbal product packed with minerals and vitamins readily soluble in water and contains a blend of *Partials spp* (leaves), *Garcina kola* (roots), *Colocynthis citrullus* (fruits) and *Linocieas nilotica* (fruits) (Oosa, 2018). It is a remedy used in the treatment of various ailments such as general body cleansing, infections, muscular problem, waist and stomach problems, joint pain; also used as a detoxifier, aid in gastrointestinal problems, pelvic and reproductive problems (Oosa, 2018).

## Materials and Method

### *Drugs*

The herbal product, Bitter Extra was purchased from a registered pharmaceutical shop in Abakaliki, Ebonyi State, Nigeria. The Bitter Extra was ascertained to have been registered with the National Agency for Food, Drug Administration and Control (NAFDAC) with Registration Number A1-1176L. The manufacture date and expiry date of the product were inspected and confirmed to be in a good time frame. The manufacturer's seal was also inspected to ascertain that its originality was intact. Each bottle contains 200 ml of the content.

### *Experimental animals*

A total number of sixteen (16) adult male Wistar rats with their initial weight ranging from 155g-230g were procured from the Animal House of the Department of Veterinary Medicine, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The animals were handled in accordance with the Guide for the Care and Use of Experimental Animals-Eighth Edition (NRC, 2011) as adopted by the Faculty of Basic Medical Sciences Research and Ethics Committee of Ebonyi State University, Abakaliki, Ebonyi State, Nigeria with ethical code number: MPC/1704/02/001. They were maintained in well ventilated poly-ethylene cages in a suitable experimental condition within room temperature and a normal light cycle (12hour light and 12hour dark) during the period of the experiment. The animals were allowed access to standard diet and drinking water *ad libitum*.

The animals were divided into four (4) experimental groups, each consisting of four rats and treated for a period of four (4) weeks as follows: Group 1 was given distilled water (Control), Group 2 was administered with 1.35ml/kg of Bitter Extra (Low dose), Group 3 was administered with 2.7ml/kg of Bitter Extra (Medium dose) whereas Group 4 took 5.4ml/kg of Bitter Extra (High dose). The graded daily doses gave the opportunity of studying the effect of the low, medium and higher doses of Bitter Extra. They were all weighed daily with BRECKNELL EPB500 Pocket Balance Digital Scale with its calibrations in grams and their weights recorded.

The Bitter Extra was administered to the animals through an orogastric tube for a period of four weeks (28 days). The animals were sacrificed by anesthetizing them in a jar containing cotton wool soaked in diethyl ether 24 hours after the last dose was administered. Blood sample were taken by simply incising the jugular vein and evacuating the blood into heparinized bottle.

The Automated Hematological Analyzer (2800 Haematology Auto-Analyzer) (Ode *et al.*, 2017) was used to analyze blood samples. Parameters analyzed were packed

cell volume (PCV), White blood cell (WBC) count, Red blood cell (RBC) count, Mean Cell hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), and Hemoglobin (Hb) concentration.

### *Statistical analysis*

The data were entered and analyzed using Statistical Package for Social Sciences (SPSS) Version 20. Students' t-test was employed and the mean was presented in Mean ±SD. P values less than 0.05 were considered to be statistically significant.

## Results

The mean body weight of the animals in the test groups were measured and compared with that of the control group to ascertain the changes associated with their body weights as shown in Table 1. The mean body weights were observed not to be statistically significant (P>0.05) among and within the test groups and the control. From the results obtained on hematological parameters, there were significant changes (P<0.05) in MCV and MCH among the groups. The Mean corpuscular hemoglobin concentration (MCHC) in both the control and the test groups were not statistically significant (P>0.05). There was marginal decrease in WBC that was dose-related in the test groups, these decrease were however significantly different between test group 4 and control (P<0.05) but not significant (P>0.05) between test groups 2 and 3 with the control. Changes in Hb, and PCV, were noticed to be significant (P<0.05) between test groups 3 and 4 when compared with the control but not significant (P>0.05) between test group 2 and control. In Red blood cell (RBC), there is high significant (P<0.05) difference between test group 4 and control but not significant (P>0.05) with test groups 2 and 3.

Table 2 indicates that the mean ± SD of groups 1, 2, 3 and 4 in MCV are 1604679.50 ±90970.50, 2019493.00 ± 83593.81,2096825.33 ± 95277.68 and 2371710.50 ± 110805.44 respectively. This table also shows that among these four groups, there were decreased significance difference in MCV (P<0.05).

Also the mean ± SD of MCH in groups 1, 2, 3 and 4 are 551493.56 ± 13771.60, 666081.63 ± 44765.14, 703001.43 ± 30877.69 and 807141.63 ± 23064.22 accordingly. The statistical difference observed among these groups were not significant (P<0.05).

MCHC presents 34.31 ± 1.55, 32.96 ± 0.86, 33.54 ± 0.73 and 34.06 ± 0.73 as mean ± SD in groups 1, 2, 3 and 4 respectively. But the statistical difference of MCHC among these four groups were significantly high (P>0.05).

*Table 1: Showing the comparison of changes in Mean Body Weight of the Rats in weeks.*

| | Groups | Mean± SD | P-value |
|---|---|---|---|
| Week 1 | G1 | 154.70±4.74 | 0.12 |
| | G2 | 189.18±30.35 | |
| | G3 | 169.98±20.44 | |
| | G4 | 196.55±32.68 | |
| Week 2 | G1 | 161.30±2.61 | 0.33 |
| | G2 | 189.33±33.51 | |
| | G3 | 172.63±26.30 | |
| | G4 | 195.05±34.96 | |
| Week 3 | G1 | 162.38±7.83 | 0.11 |
| | G2 | 201.57±28.53 | |
| | G3 | 190.67±10.93 | |
| | G4 | 198.53±29.46 | |
| Week 4 | G1 | 163.83±8.14 | 0.30 |
| | G2 | 198.20±34.69 | |
| | G3 | 184.10±13.79 | |
| | G4 | 189.20±28.87 | |

This table shows that among these four groups in weeks 1, 2, 3, and 4 there were no significance differences in weight (P>0.05).

*Table 2: Comparison of MCV, MCH and MCHC between groups of the experimental animals and control.*

| | Groups | Mean ±SD | P-value |
|---|---|---|---|
| MCV (fl/c) | G1 | 1604679.50±90970.50 | 0.0001 |
| | G2 | 2019493.00±83593.81 | |
| | G3 | 2096825.33±95277.68 | |
| | G4 | 2371710.50±110805.44 | |
| MCH (pg/cell) | G1 | 551493.56±13771.60 | 0.0001 |
| | G2 | 666081.63±44765.14 | |
| | G3 | 703001.43±30877.69 | |
| | G4 | 807141.63±23064.22 | |
| MCHC (g/dl) | G1 | 34.31±1.55 | 0.453 |
| | G2 | 32.96±0.86 | |
| | G3 | 33.54±1.09 | |
| | G4 | 34.06±0.73 | |

## DISCUSSION

In some parts of the world and indeed Nigeria, herbal medicines are employed in the management of various diseases. Plants and herbs are sources of many efficacious and potent drugs (Iwu, 2014; Gurib-Fakim, 2006) and herbal medicine constitute a larger proportion of the health care needs of developing countries (Hosseinzadeh *et al.*, 2015; Mahomoodally, 2013). However, despite the inherent benefits of herbal medicines, and the perceived safety and non-toxic nature, available literature have shown their role in the aetiology of various forms of complications (Knoss, 2017; Obidike and Salawu, 2013),

making it imperative to investigate their potential adverse effects (Arome and Chinedu, 2014). By determining the effects of Bitter Extra on the haematological parameters and body weight, we seek to establish its safety and provide recommendations on the safe use of this herbal product for medicinal purposes.

*Table 3: Showing the multiple comparison of the hematological variables*

| | Experimental Groups | | Mean±SD | P-value |
|---|---|---|---|---|
| WBC ($mm^3$) | G1 | G4 | 625.00±192.46 | 0.037 |
| | | G3 | 558.33±207.88 | 0.090 |
| | | G2 | 391.67±207.88 | 0.294 |
| Hb (g/dl) | G1 | G4 | 1.68±0.47 | 0.023 |
| | | G3 | 2.69±0.51 | 0.002 |
| | | G2 | 0.56±0.51 | 0.703 |
| PCV (%) | G1 | G4 | 5.25±1.54 | 0.029 |
| | | G3 | 6.67±1.66 | 0.011 |
| | | G2 | 1.00±1.66 | 0.929 |
| RBC (l) | G1 | G4 | -57.50±6.61 | 0.000 |
| | | G3 | 0.00±7.14 | 1.000 |
| | | G2 | -20.00±7.14 | 0.075 |

This multiple comparison table reveals that there were significant differences (P>0.05) in WBC, Hb, PCV and RBC.

Available evidence has shown that the consumption of toxic herbal products can cause alterations in the hematological profile (Sani *et al*., 2009; Zahmati and Saljooghi, 2016) and drugs associated with toxic effect could cause organ damage and significant alteration in haematological biomarkers (Arome and Chinedu, 2014). The determination of hematological parameters provides physiological information on a proper blood assessment in the body. In this study, rats administered with Bitter Extra show no significant changes in their body weights, and therefore suggests the fact that Bitter Extra has no adverse effect on the body weight. This lack of significant changes in the body weights further supports the idea of potential safety of the product. However, it should be noted that a change in the body weight is an uncomplicated and sensitive index to study the detrimental effects of drugs and chemicals (Bailey *et al*., 2004). In a general term, adverse effect of a drug could lead to abnormalities in the body weight and a decrease in body weight could indicate a substantial degree of damage while a reduced body weight gain represents only a mild form of damage (Michael *et al*., 2007; Piao *et al*., 2013). Furthermore, body weight is a very sensitive indicator of adverse drug effect and any subtle alteration is of significant importance for further investigation (Piao *et al*., 2013; Michael *et al*., 2007).

In this study, there's an increased level of free WBC count and Hb concentration which may be associated with inflammation arising from assault on vital organs. In fact, increased level of WBC count (specifically leukocytes), has been shown to correlate well with C-reactive protein (CRP), an important marker of inflammation (Hemelrijck *et al*., 2011; Arika *et al*., 2016). The general lack of significant changes in WBC count in lower doses suggests an anti-inflammatory property of Bitter Extra when taken moderately. The significant increase in WBC count at 5.4 ml/kg when compared to 1.35ml/kg and 2.7ml/kg may further explain the adverse effect of the Bitter Extra as the dose increases.

The mean cell volume (MCV) is an indication of the percentage of the red cells in the total blood, and provides an indication of the oxygen carrying capacity or efficiency of the RBC. The observed increase in MCV could be an indication of clinical condition associated with high MCV (Arika *et al*., 2016; Leach, 2014; Hall, 2016). This significant increase in MCV as the dose increases suggests there's no iron deficiency anaemia, lack of significant changes in MCHC. However, a significant reduction in MCV can be caused by an insufficient production of healthy RBC with normal size and shape, an increased number of WBC, deficiencies in vitamin or mineral and overhydration (Arika *et al*., 2016; Leach, 2014; Hall, 2016). In this study, the RBC count and Hb concentration at lower doses appear normal with no significant changes when compared with control but with an increased significant changes at higher doses, and this suggests that Bitter Extra at higher doses may have caused an insufficient production of healthy RBC with normal size and shape. Specifically, it appears that the RBC that were produced has a higher proportion of erythrocytes with smaller sizes (as indicated by MCV), suggesting iron deficiency hypochromic microcytic anemia (Arika *et al*., 2016; Leach, 2014; Hall, 2016). Polycythemia could result from hyperosmotic conditions arising from high dosage of toxic agents (Arika *et al*., 2016; Leach, 2014). On the other hand, MCV measures the average volume or size of RBC (Arika *et al*., 2016; Leach, 2014) and a low MCV (microcytic) is consistent with anaemia and thalassaemia syndromes, and an elevation (macrocytic) could be a reference to deficiencies in vitamin B12 and folate (Arika *et al*., 2016; Leach, 2014).

## Conclusion

The consumption of Bitter Extra as herbal product may be assumed to be safe judging from the lack of serious changes in the body weight even in high doses. However, the observed increased significance in some haematological parameters show the potential of the herbal product to effect adverse changes at higher doses. There is greater need to take caution and avoid abuse and indiscriminate use of Bitter Extra. This is more important

going by the potential for cumulative adverse effects from continuous usage.

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