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Assessment of Haematological Changes in Albino Wistar Rats Following Oral Administration of Graded Doses of Alcoholic Polyherbal Formulation (Action Bitter)

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Abstract: Medicinal plants can be used for the synthesis of useful drugs. The aim of this study was to evaluate the haematological changes in albino wistar rats treated with graded doses of alcoholic polyherbal formulation (action bitter). Albino wistar rats were assigned into four groups: a control group (A) that received only distilled water while the 3 test groups B, C and D received 100, 200 and 300 mg kg^{-1} dose of action bitter respectively, daily for 3 months. Blood sample was collected from all groups and Packed Cell Volume (PCV), White Blood Cell (WBC) count, platelet count were determined. Action bitter was also analysed for heavy metal content. One hundred and twenty wistar rats, made up of 60 (50%) males and 60 (50%) females were used for the study. Among the males, PCV progressively increased with a mean±SD of 42.3±2.0% in the control group to 45.3±1.2%, 49.7±2.1% and 48.7±3.1 among groups B, C and D, respectively. Similarly, among female rats, mean PCV progressively increased from 44.0±0.0% in the control group to 46.0±2.8, 47±0.0 and 45.9±1.6% among groups B, C and D, respectively. In the same way, mean platelet count significantly increased progressively among male rats from $156±57×10^9$ L^{-1} in the control group to 232±35, 257±41 and $291±10×10^9$ L^{-1} among groups B, C and D ($p<0.05$). Females also showed similar result with progressive platelet increase from $228±17×10^9$ L^{-1} in the control group to 240±51, 226±40 and $236±51×10^9$ L^{-1} among group B, C and D, respectively. Additionally, WBC progressively decreased among males from mean value of $12.9±3×10^9$ L^{-1} in the control group to 12.3±2, 10.1±1 and $11.5±2×10^9$ L^{-1} among groups B, C and D, respectively. Females also had progressively lower WBC count from mean value of $10.9±3×10^9$ L^{-1} among the control group to 10.2±4, 9.2±2 and $8.2±2×10^9$ L^{-1} among groups B, C and D, respectively. Action bitter contain iron, zinc, copper and chromium at the level of 61.2, 1.74,

0.89 and 0.02 mg kg^{-1}, respectively. Administration of action bitter to Albino wistar rats increased PCV and platelet count but decreased WBC count. Essential heavy metal content of action bitter includes iron, zinc, copper and chromium. Further studies are needed to evaluate the therapeutic effect of action bitter in management of anaemia and thrombocytopeania.

INTRODUCTION

Medicinal plants have been defined as those plants which contain in one or more of their organs, substances that can be used for the synthesis of useful drugs[1]. The 80% of African population use some form of traditional herbal medicine[2] and herbal medicine is still the most abundant, affordable, reliable, trusted and well understood form of health care in virtually all African villages[3]. Polyherbal medicines are administered in most disease conditions over a long period of time without proper monitoring and consideration of toxic effects that might results from such prolonged usage[4]. The increase demand for herbal products coupled with the erroneous impression by the people that herbal products are natural and thus less harmful to the body has brought concern and fear over the quality, efficiency and safety of some of the available natural herbs. Current trends showed that the inability to afford modern medical healthcare in developing countries has forced patients to seek traditional medical attentions[5].

Bitters are made up of numerous groups of chemical compounds extracted from the herbs and roots (medicinal plants) that have common characteristic of a bitter taste and act to increase the vital energy centres in the body[6-8] and they contain a variety of chemical substances of secondary metabolism that have physiological and pharmacological activities. Bitters are traditionally an alcoholic preparation flavoured with botanical matter such that the end result is characterized by a bitter, sour or bitter-sweet flavour. Bitters are generally claimed to be effective in curing all allergic, metabolic and immunological conditions where the diagnosis points to a fault in the digestive process, improving immunity, anaemia, wound healing and blood clotting by increasing the population in tissues, of red blood cells, white blood cells and platelets[9]. It is also helpful in inflammatory conditions of the gastrointestinal tract (colitis, crohn's disease, non specific inflammation)[9]. Bitters are said to have anti-inflammatory, antibiotics and antifungal properties[9] as well as enhance good digestion of fats and oils and proper functioning of the liver as regards to excretion, reduce accumulated fat (triglycerides) and cholesterol levels therapy conferring on it hypolipdaemic properties[9]. Bitters promote the production and release of pancreatic enzymes and so have hypoglycaemic properties[9]. Herbs have been reported to be of utmost advantage for the management of obesity and over weight[10, 11]. Alcoholic polyherbal formulation is a highly patronized herbal medicine consumed by a large African population either for recreational or medicinal purposes. However, due to disparity of several studies on the health implications and benefits of these highly patronized alcoholic polyherbal products, there is need to evaluate the haematological effects on albino wister rats treated with graded doses of action bitter (herbal formulation) in a dosage-duration dependent manner. This will help to give an insight on its haematological effects on humans and help in educating the general public on the benefits and the diseases that may arise from consumption of such formulation. The objective of this study was to evaluate the haematological parameters of albino wistar rats treated with graded doses of action bitter (herbal formulation).

MATERIALS AND METHODS

Study design: This was a prospective study carried out between November 2018 to November 2019.

Substance of study: A preliminary survey was conducted to know the most widely consumed alcoholic polyherbal formulations. This survey was at different local kiosk, supermarkets and street hawkers within Abakaliki, Ebonyi State Nigeria. The most widely consumed alcoholic polyherbal formulation observed was action bitter. Several bottles of action bitter were purchased from a reputable pharmacy and were stored at room temperature throughout the period of the experiment. All the albino wistar rats that were used for all stages of this study were handled in strict compliance with international guidelines as prescribed by the Canadian Council on Animal Care and Use of Laboratory, 1984.

Experimental animals: Wistar albino rats of comparable weights and sizes were procured from the animal house of the Department of Clinical Pharmacology, College of Medicine, University of Uyo, Akwa Ibom State Nigeria. The animals were assigned into four groups: a control group (A) and three test groups based on the concentration of action bitter received into groups B, C and D. These groups were subdivided into A1-D1, A2-D2 and A3-D3 representing experimental durations of one month (Day 28), two months (Day 56) and three months (Day 84), respectively. The rat was allowed to acclimatize for two weeks and was fed ad libitum during this period, with water and chukun chelated grower feed.

Substance administration: All the animal groups were fed with chukun chelated grower feed plus water given *ad libitum*. However, group A (control) received distilled water only, test groups B-D received different doses per body weight of action bitter orally using oro-gastric gavage. Low dose (B) = 100 mg kg^{-1}, Intermediate dose (C) = 200 mg kg^{-1}, High dose (D) = 300 mg kg^{-1} .

Blood sample collection and preparation at the end of each month: The last doses of the selected polyherbal formulation were administered on the morning of the 28th day of every month and all meals were stopped by 7 pm on the 28th day of every month. After an overnight fast, the animal was sacrificed by administering chloroform anaesthesia and dissected. Blood samples were collected from the lateral aspect of the eye using heparinized capillary tubes and also into already labeled EDTA bottles and then mixed by gentle inversion.

Haematological parameters: Blood samples from experimental animals collected into Ethylene Diamine Tetra Acetic Acid (EDTA) bottles were used to estimate Packed Cell Volume (PCV), Total White Blood Cell Count (TWBC) and Platelet Count. Blood for PCV estimation was drained into labeled haematocrit capillary tubes to two-third full with one end of the capillary tube sealed with plasticine. The tubes were placed in microhaematocrit centrifuge and spun at 10,000 rpm for 5 min. Thereafter, the PCV was read with microhaematocrit reader and the readings were expressed in percentage. For the WBC, the blood in the vial with EDTA was carefully drawn to 0.5 mark on white cell pipette and mixed thoroughly after it has been covered with finger tips. The blood was diluted with WBC diluting fluid to lyse red cells. At an angle of 45°, the blood in the vial was introduced into the improved Neubauer counting chamber (Haematocytometer) (after placing cover slip on the Neubauer chamber) without allowing the fluid to overflow. The chamber was then placed on the microscope stage and allowed to settle for 10 min, so that using the 4 mm objective and ×10 eye piece; all the cells were counted including cell touching the borderline on the top and right hand side. The actual number of white cells counted was reported as number of white cells counted $\times 10^9$ L^{-1}.

For Platelet count, blood was diluted with filtered solution of 1% aqueous ammonium oxalate reagent at the ratio of 1 in 20 which lysed the red cells. Hence, 380 uL of filtered ammonium oxalate diluting fluid was added to 20 µL (0.02 mL) of well mixed anti-coagulated venous blood and mixed properly. Neubauer counting chamber was filled with the well mixed sample and was left undisturbed for 10 min. Platelets were counted microscopically in the small central square of improved Neubauer ruled counting chamber using ×40 objectives and the number of platelet per liter of blood calculated. The actual number of platelet counted was reported as number of platelet counted $\times 10^9$ L^{-1}.

Statistical analysis: Data generated from this study was analysed using Statistical Package for Social Sciences (SPSS) software, version 20. Descriptive statistics was used to generate proportions and percentages, means and standard deviation. Student t-test was used to compare means. $p<0.05$ were considered significant.

Ethical issues: Approval for this study was gotten from the Research and Ethics Committee of the University of Nigeria, Enugu Campus. All the albino wistar rats that were used for all stages of this study were handled in strict compliance with international guidelines as prescribed by the Canadian Council on Animal Care and Use of Laboratory, 1984.

RESULTS AND DISCUSSION

One hundred and twenty wistar rats, 60 males and 60 females of comparable weights and sizes were used for this study.

Packed Cell Volume (PCV): Among male and female rats, packed cell volume progressively increased as the concentration of action bitter increased from 100-200 mg kg^{-1}, with slight decrease in PCV at the concentration of 300 mg kg^{-1} (Table 1 and Fig. 1).

Total White Blood Cell Count (TWBC): Among male rats, white blood cell count decreased with increase in concentration of action bitter with a mean value of 12.9×10^9 L^{-1} in control group to 11.5×10^9 L^{-1} among rats that received 300 mg kg^{-1} of action bitter (Table 2 and Fig. 2). Likewise, among female rats, WBC count progressively decreased from 10.9×10^9 L^{-1} in the control group to 8.2×10^9 L^{-1} among rats that received 300 mg kg^{-1} of action bitter (Table 2 and Fig. 2).

Platelet count: Among male rats, platelet count increased with increase in the concentration of action bitter with an average of 156×10^9 L^{-1} among the control group to an average of 291×10^9 L^{-1} among rats that received 300 mg kg^{-1} body weight of action bitter (Table 3 and Fig. 3). Similar result was also seen among female rats with progressive increase in the average platelet count from 228×10^9 L^{-1} in the control group to 236×10^9 L^{-1} among rats that received 300 mg kg^{-1} body weight of action bitter (Table 3 and Fig. 3).

Table 1: Packed Cell Volume (PCV) of male and female albino wistar rats treated with action bitter

	PCV (%)							
	Male				Female			
Duration	Control	100 mg kg^{-1}	200 mg kg^{-1}	300 mg kg^{-1}	Control	100 mg kg^{-1}	200 mg kg^{-1}	300 mg kg^{-1}
1st Month	42.0±2.8	46.0±2.8	52.0±2.8	48.0±2.8	48.5±2.1	42.0±2.8	40.0±0.0	42.0±1.4
2nd Month	40.5±0.7	44.0±2.8	48.0±2.8	46.0±2.8	40.0±1.4	43.0±4.2	39.0±1.4	39.0±0.0
3rd Month	44.5±2.1	46.0±1.4	49.0±1.4	52.0±0.0	63.0±1.4	56.0±7.1	59.0±7.1	43.0±14.1
Average	42.3±2.0	45.3±1.2	49.7±2.1	48.7±3.1	44.0±0.0	46.0±2.8	47.0±0.0	45.9±1.6
t-value	-	-2.233	-4.378	-2.995	-	0.183	0.616	0.590
p-value	-	0.089	0.012	0.040	-	0.864	0.571	0.587

Table 2: Total White Blood Cell Count (WBC) of male and female albino wistar rats treated with action bitter

	WBC							
	Male				Female			
Duration	Control	100 mg kg^{-1}	200 mg kg^{-1}	300 mg kg^{-1}	Control	100 mg kg^{-1}	200 mg kg^{-1}	300 mg kg^{-1}
1st Month	14750.0±353.5	14250.0±1060.7	8750.0±2474.9	11800.0±424.3	13950.0±1484.9	15000.0±141.4	10950.0±2757.7	9350.0±919.2
2nd Month	14500.0±707.1	12100.0±424.3	10900.0±2687.0	9500.0±2121.3	9800.0±16971.1	8600.0±848.5	9050.0±70.7	9750.0±1343.5
3rd Month	9500.0±707.1	10400.0±565.7	10750.0±1060.7	13250.0±1712.8	9100.0±141.4	7150.0±919.2	7500.0±707.1	5550.0±2757.7
Average	12916.67±2961.6	12250.0±1929.4	10133.3±1200.3	11516.7±1891.0	10950±2621.5	10250±4177.0	9166.7±1728.0	8216.7±2318.0
t-value	-	0.327	1.509	0.301	-	0.246	0.984	1.353
p-value	-	0.760	0.206	0.528	-	0.818	0.381	0.248

Table 3: Total platelet count of male and female albino wistar rats treated with action bitter

	Platelet							
	Male				Female			
Duration	Control	100 mg kg^{-1}	200 mg kg^{-1}	300 mg kg^{-1}	Control	100 mg kg^{-1}	200 mg kg^{-1}	300 mg kg^{-1}
1st month	96500.0±0606.6	271000.0±12727.9	303000.0±4953.3	283000.0±9899.5	212500.0±606.6	292500.0±88388.3	265000.0±7071.1	294000.0±22627.4
2nd month	210000.0±28284.3	219000.0±12727.9	245000.0±7071.1	288000.0±2828.4	247500±17677.6	240000±56568.5	228000.0±31112.7	216500.0±17677.7
3rd month	160000.0±14142.1	205000.0±7071.1	223500.0±4949.7	303000.0±9899.5	225000.0±29698.5	190000.0±28284.3	185000.0±7071.1	199000.0±1414.2
Average	155500.0±56883.7	231666.7±34775.47	257166.7±41122.8	291333.3±10408.3	228333.3±17736.5	240833.3±51255.08	226000±40037.48	236500±50559.37
t-value	-	-3.006	-3.420	-6.138	-	-0.431	0.044	-0.379
p-value	-	0.013	0.007	0.001	-	0.675	0.903	0.713

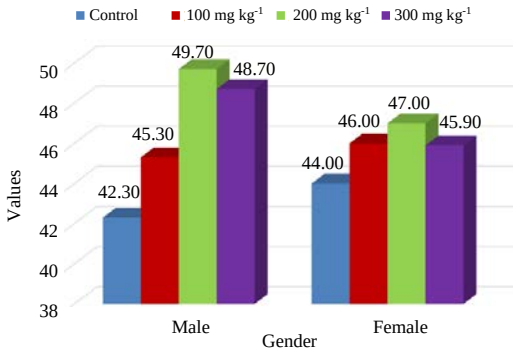

Fig. 1: Chart showing the Packed Cell Volume (PCV) of the male and female albino wistar rats treated with action bitter

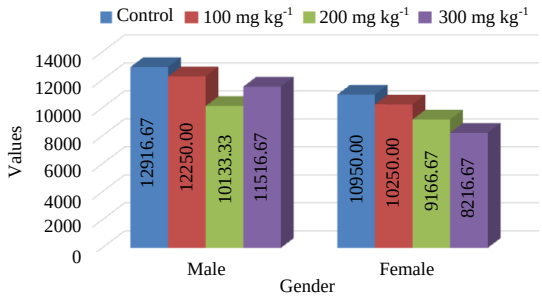

Fig. 2: Showing total White Blood Cell Count (TWBC) male and female albino wistar rats treated with action bitter

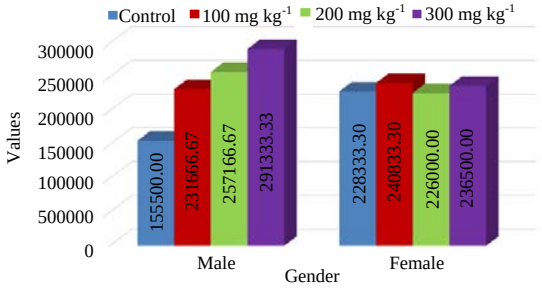

Fig. 3: Chart showing the total platelet count of male and female albino wistar rats treated with action bitter

Heavy metals: Analysis of action bitter for heavy mental content showed that it contain iron at the concentration of 61.2 mg kg^{-1}, zinc 2.74 mg kg^{-1} and other essential heavy metals and lack toxic metals such as lead and arsenic (Table 4).

The haematologic indices obtained from this study when control groups were compared with the result of male and female wistar rats treated with different dosages of action bitter showed that Packed Cell Volume (PCV) increased significantly among males ($p<0.05$). However, the increase in PCV was not significant among female rats ($p>0.05$). Previous studies by Adeneye and Benebo[12] also reported increased PCV with administration of polyherbal tonic tea in rats. It was also reported to cause elevated platelet count, total leukocyte counts and lymphocyte differentials while causing significant

Table 4: Heavy metal analysis result of action bitter showing it's heavy metal content

Heavy metals	Action bitter content (mg kg^{-1})	FAO/WHO recommended value (g kg^{-1})
Iron	61.20	43.00
Zinc	1.74	3.00
Copper	0.89	2.00
Chromium	0.02	0.08
Selenium	ND	0.2
Lead	ND	0.01
Arsenic	ND	0.01

ND = None Detected; FAO = Food and Agricultural Organization

suppression of granulocyte differentials in dose-related fashion. However, work done by Ebenebe *et al*.[13] contradict our findings. They reported that there was a significant decrease in PCV and total WBC with some herbal formulations (Gracinia kola and Alomo bitters, respectively). The result of this study indicated that action bitters did not exhibit haematological toxicity.

Elevated PCV caused by action bitter may be due to the fact that it was also found to contain iron which is one of the important elements required for erythropoiesis. Administration of iron has been reported to enhance erythropoiesis with elevated PCV while iron deficiency is associated with anaemia and decreased PCV. However at the dose of 300 mg kg^{-1} of action bitter, the PCV dcreased a bit for both male and female rats. This suggests that higher dose of action bitter above 200 mg kg^{-1} over time may have negative effect on erythropoiesis[14]. Zinc may also have contributed to the elevated PCV. Action bitter was also found to contain zinc which is an antioxidant that reduces red cell hemolysis and thereby prolonging red cell survival with consequent increase in PCV[15]. Copper was also found as another content of action bitter. Copper is an essential trace element which has been reported to play important role in iron absorption and metabolism. It has been reported that copper deficiency in experimental animals results in anaemia which has many characteristics of bone marrow failure with ineffective erythropoiesis[16]. Chromium have also been reported to have synergistic effect to iron and lower concentrations have been found in blood of anaemic patients when compared to control subjects[17].

This study also found that the mean total White Blood Cell Count (WBC) decreased in the test group compared to the control groups and the decrease tend to be more with increase in dose of action bitter among both male and female rats, though the finding was not significant. This is similar to the study done by Showande *et al*.[18+] who reported reduction in WBC in male Wistar rats with administration of polyherbal formulations (Fidson bitter and daily living bitter). The suppressive effect of action bitter on white blood cell count may be useful in management of conditions that lead to excessive white cell count such as leukaemia. It could also lead to immune suppression on the other hand.

This study also found that there was an increase in the platelet count in both male and female rats treated with action bitters compared to the control group with a significant increase ($p<0.05$) in males. However, the increase was not significant in females ($p>0.05$). The result of this study suggests that action bitter may have platelet augmentation activity.

Heavy metals content analysis of action bitters showed that it has high content of iron above WHO recommended value and lower content of zinc, copper and chromium below WHO recommended value. These metals are referred to as nutritional or essential heavy metals. They play important role in haemopoiesis in addition to other functions. Toxic or non-essential heavy metals such as mercury, cadmium and lead were noted to be absent in action bitters. This shows that action bitter is safe for consumption.

CONCLUSION

Administration of action bitter to albino wistar rats caused elevated PCV and platelet count but decreased white blood cell count. Action bitter contain essential heavy metals such as iron, zinc, copper and chromium but lack toxic metals such as mercury, arsenic and lead and is therefore safe for consumption.

RECOMMENDATIONS

Further studies are needed to evaluate the actual therapeutic effect of action bitter in the management of anaemia and thrombocytopeania.

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