



## **Effect of Chronic Administration of *HRT 123* Herbal Formulation on Some Haematological Indices and Serum Biochemical Markers in Wistar Rats**

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### **Authors' contributions**

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

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### **ABSTRACT**

Herbal preparations continue to be the mainstay of treatments for diverse medical conditions worldwide especially in sub-Saharan Africa and other resource-constrained areas. The preparations are often taken for a prolonged period due to the chronic nature of some of the medical conditions (such as cancer, HIV/AIDS) being managed. Consequently, the safety of the preparations needs to be carefully considered. One such preparation, traditionally claimed to have immune boosting potentials in patients with conditions associated with decreased immune function and in chronically ill and debilitated patients is the herbal formulation *HRT 123*. The study evaluated effects of chronic administration of *HRT 123* aqueous extract on some haematological indices and serum biochemical markers. Five groups of six Wistar rats each were used. Group 1 was administered distilled water only. Groups 2 to 5 were administered 50, 100, 200 and 400 mg/kg respectively of *HRT 123* aqueous extract orally for 90 days. Animals were sacrificed at the end of extract administration. The blood was analyzed for haematological indices, parameters of liver (AST, ALT, total protein and albumin) as well as kidney (sodium, potassium, chloride

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and creatinine) function. There were no statistically significant changes ( $P > 0.05$ ) in haematological indices as well as serum biochemical parameters and electrolytes in treated animals relative to control. These results suggest that *HRT123* may not have adverse and deleterious effects on functions of organs such as the liver and kidneys when administered over a prolonged period.

**Keywords:** Herbal tea; polyherbal formulation; chronic toxicity; serum biochemical markers.

## 1. INTRODUCTION

Herbal preparations continue to be the main stay of treatments for diverse medical conditions worldwide especially in sub-Saharan Africa and other resource constrained areas of the world. Some of the preparations are often taken for a prolonged period of time due to the chronic nature of some of the medical conditions (such as cancer, HIV/AIDS) being managed. In many cases, it is the terminal nature of some of the conditions being treated as well as the absence or inaccessibility of orthodox management that prompts and promotes the use of these herbal formulations [1]. With respect to these chronic and debilitating conditions, some patients use the herbal preparations concurrently with their conventional medicines as has been reported at an antiretroviral clinic in South West Nigeria [2]. For some of these conditions, the preparations are touted to be used as immune boosters or cleansers [2,3].

The point of concern, however, is that patients often fail to inform their health care providers or outrightly deny such usage [4]. As a result, the usage may be poorly monitored thereby leading to increased incidences of unwanted effects and interactions with other co-administered drugs [5]. In the face of these shortcomings, there is the need to scientifically evaluate the claims relating to many herbal preparations with a view to authenticating the claims to their efficacy and safety. This is more so as many of these preparations are to be used in chronic conditions [6].

*HRT 123* is a polyherbal preparation containing plant parts/components from *Aframomum melegueta*, *Monodora myristica*, *Xylopiya aethiopica*, *Gongronema latifolium*, *Allium sativum*, *Garcinia kola* and *Viscum album*. It is used as an herbal tea in conditions associated with immuno-suppression and chronically debilitated states where it is claimed to have immune boosting potentials. This study evaluated the effect of chronic administration of *HRT 123* aqueous extract on some haematological indices and serum biochemical markers. These represent key parameters that

may be affected following prolonged administration of a drug.

## 2. MATERIALS AND METHODS

### 2.1 Preparation of Extract

*HRT 123* is a coarse powder packed as 25 tea bags in a bigger 50 gmpacket. The extract was prepared from the coarse powder as earlier described by Falang et al. [7]. In the procedure, 500 gm of finely divided *HRT 123* powder was extracted with 5 litres of hot water (solvent to herb ratio of 10:1) over 24 hours with occasional shaking. The filtrate was then passed through sieves of decreasing pore sizes (800, 500 and 150  $\mu\text{m}$ ) and finally through Whatman (No. 1) filter paper. The final filtrate was concentrated to dryness in an oven maintained at 40°C. The dark green solid residue obtained was kept in an airtight container in a refrigerated condition until required for use.

### 2.2 Experimental Animals

Thirty freshly weaned Wistar rats of either sex weighing between 150 and 200 gm raised at the Animal House Unit, Department of Pharmacology, Faculty of Pharmaceutical Sciences of the University of Jos were used for the study. The Institutional Animal Care and Use Committee approved the protocols for the experiments. Animals were handled under the guidelines of ETS 123 [8] and Guiding Principles in the Use of Animals in Toxicology [9]. The animals were appropriately housed in a well-ventilated room at controlled temperature ( $25 \pm 2^\circ\text{C}$ ) under natural dark-light cycle. They had free, uninhibited access to feed and water throughout the duration of the study.

### 2.3 Chronic Toxicity

Chronic toxicity was evaluated according to World Health Organization guidelines [10]. In the procedure, five groups of six rats each were used. To group one which was the control was administered distilled water only. To groups two to five were administered 50, 100, 200 and 400

mg/kg respectively of the extract which had been freshly reconstituted in distilled water. In all cases, the extract was administered by oral gavage once daily over a period of ninety days. Animals were left free of treatment for 24 hours before sacrifice and were humanely sacrificed after being anaesthetized with chloroform through the open-drop procedure described by Risling et al. [11].

#### 2.4 Evaluation of Haematological Indices and Serum Biochemical Parameters

Blood was collected from the animals through cardiac puncture using the method described by Parasumaran et al. [12]. Blood that was to be used for evaluation of serum biochemical parameters was collected in plain sample bottles. This was allowed to clot, centrifuged at 4000 rpm for ten minutes and the serum collected. The serum was stored at -20°C until the evaluations which was carried out on the blood chemistry autoanalyzer (Cobas C311). Blood that was to be used for haematological investigations was collected in ethylenediaminetetraacetic acid (EDTA) bottles and the evaluations were carried

out on the haematology auto analyzer (Mindray BC 3200).

#### 2.5 Data Analysis and Presentation

Data were analyzed using one-way analysis of variance (ANOVA) and student's t-test with the aid of SPSS statistical software Version 20 [13]. Differences in means were considered to be significant when  $p < 0.05$ .

### 3. RESULTS

Results for the effects of HRT 123 aqueous extract on haematological indices are presented in Table 1 and Table 2. In Table 1, the platelets showed significant decreases compared with control at the 100 and 400 mg/kg dose levels ( $p < 0.05$ ). The white blood cells, neutrophils, absolute neutrophil count, absolute lymphocyte count all showed non-significant increases compared with control ( $p > 0.05$ ). Table 2 shows that the red blood cell indices were stable with only the Mean Corpuscular Haemoglobin and Mean Corpuscular Volume showing increases which were significant at the 100 mg/kg body weight dose level compared with control group.

**Table 1. Effect of chronic administration of HRT 123 extract on White Blood Cells and differentials in rats**

Parameters	Treatment (mg/kg)				
	Control	Extract 50	Extract 100	Extract 200	Extract 400
Total WBC (X 10 <sup>9</sup> /L)	13.72±5.05	16.40±6.30	21.90±2.50	12.58±3.22	16.67±2.16
Neutrophils	30.83±5.11	27.25±5.12	25.00±6.00	32.00±1.00	31.33±4.92
ANC	4.08±1.11	4.43±2.10	5.35±0.75	3.10±0.10	5.20±1.28
Lymphocytes	60.00±5.69	62.50±3.35	66.00±7.00	58.50±1.50	60.00±6.98
ALC	8.43±3.72	10.25±3.80	14.55±3.00	5.65±0.05	9.97±1.51
Monocytes	7.17±0.98	8.00±6.48	7.00±1.41	7.50±0.71	7.00±2.00
Eosinophils	2.00±0.00	2.00±1.58	2.00±0.00	1.50±0.50	1.67±0.47
Platelets	1072.80±128.99	949.33±93.20	870.00±66.00*	983.33±131.49	808.67±33.63*

Values represent mean ± SEM; n = 6; \*significantly different compared with control. WBC = White Blood Cells; ANC = Absolute Neutrophil Count; ALC = Absolute Lymphocyte count

**Table 2. Effect of chronic administration of HRT 123 extract on RBCs and RBC indices in rats**

Parameters	Treatment (mg/kg)				
	Control	Extract 50	Extract 100	Extract 200	Extract 400
PCV (%)	39.08±1.65	37.75±3.13	38.90±1.60	39.08±1.03	38.80±1.47
RBC (X 10 <sup>12</sup> /L)	8.83±0.49	8.38±0.59	8.23±0.28	8.43±0.16	8.50±0.62
Hb(g/dL)	13.75±0.69	13.50±1.03	13.55±0.85	13.98±0.60	13.60±0.85
MCHC (g/dL)	35.13±0.88	35.75±0.34	34.75±0.75	35.73±1.01	34.97±0.88
MCH (pg)	15.52±0.36	16.05±0.34	16.40±0.50*	16.00±0.35	15.97±0.32
MCV (fL)	44.30±1.19	45.05±0.87	47.30±0.30*	44.95±2.02	45.87±1.51

Values represent mean ± SEM; n = 6; \*significantly different from control ( $p < 0.05$ )  
PCV = Packed Cell Volume; RBC = Red Blood Cells; Hb = Haemoglobin; MCV = Mean Cell Volume;  
MCHC = Mean Cell Haemoglobin Concentration; MCH = Mean Cell Haemoglobin

**Table 3. Effect of chronic administration of HRT 123 extract on some Serum Biochemical markers in rats**

Parameters	Treatment (mg/kg)				
	Control	Extract 50	Extract 100	Extract 200	Extract 400
AST (IU/L)	32.50±5.05	24.20±5.85	37.20±10.37	29.83±8.38	30.75±3.77
ALT (IU/L)	9.67±6.67	13.00±11.92	7.40±5.12	15.50±9.07	6.75±3.27
Total Protein (g/dL)	82.67±8.20	68.00±8.69	72.80±10.03	80.17±11.38	77.00±12.98
Albumin (g/dL)	33.50±2.87	31.00±2.28	32.60±5.46	32.33±2.87	31.75±0.83
Chloride (mmol/L)	75.83±4.41	77.20±4.35	74.60±4.80	74.83±4.30	77.75±5.00
Potassium (mmol/L)	5.98±0.60	5.86±1.14	7.52±2.73	5.68±0.89	5.18±0.25
Sodium (mmol/L)	138.68±3.94	139.60±6.50	139.20±3.49	136.67±4.85	136.50±4.98
Creatinine (µmol/L)	104.33±23.22	79.00±10.06	98.80±24.59	81.50±16.15	77.25±18.99

Values represent mean ± SEM; n = 6; There was no significant difference with control ( $p > 0.05$ )

AST = Aspartate aminotransferase; ALT = Alanine aminotransferase

Results for serum biochemical markers are presented in Table 3. Liver function parameters (AST, ALT, Total protein and Albumin) were not significantly different in treated animals compared with control group as the dose of the extract increased. Similarly (chloride, potassium, sodium, creatinine) were not significantly different in treated animals compared with control.

#### 4. DISCUSSION AND CONCLUSION

Chronic toxicity studies provide information on possible health hazards that may arise with repeated long term exposure to a chemical or an agent over a considerable part of the lifespan of the species used. These types of studies provide information on the toxic effects of the substance, implicated target organs and the possibility of accumulation [14]. Studies such as these are important in the evaluations of agents that will be used in disease conditions that are chronic and will be managed over a protracted period of time and in which there may be compromised organ function and abnormalities in key systems.

Chronic administration has no significant adverse effects on total and differential white blood cell counts. The platelets were progressively reduced, reaching significant levels at the 200 and 400 mg/kg dose levels ( $p < 0.05$ ). With regards to the red blood cell parameters and indices, only the Mean Cell Volume and Mean Cell Haemoglobin significantly ( $p < 0.05$ ) increased at the 100 mg/kg dose level. But of note is that these were not particularly dose-dependent as to lead to an inference of a toxic effect as reported by other authors under similar circumstances [15]. Furthermore, macrocytosis as reflected by an elevated Mean Cell Volume (see table 2) may not always be associated with a pathologic process or condition and close to

60% of patients present without associated anaemia. No complications arise from it as an isolated finding but its identification can provide important information regarding the presence of an underlying disease state [16]. Thus MCV values above normal may require investigation to identify the cause.

Chronic administration of HRT 123 aqueousextract did not show any deleterious or toxic effect with respect to indices of liver functions (AST, ALT, total protein and albumin) as well as renal functions (sodium, potassium, chloride and albumin). The purpose of an evaluation such as this is to provide information that will validate the claimed ethnomedicinal uses of an herbal product or preparations such as HRT 123. These results suggest that the product may be safe following prolonged oral administration with little likelihood of deleterious effects on the haematological system; the kidney and liver function indices in the conditions associated with decreased immunity in which it may be used. However further evaluations may be necessary to elucidate the mechanisms through which the likely immune-boosting potential is elaborated.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

The institutional Animal Care and Use Committee approved the protocols for the experiments. Animals were handled under the guidelines of ETS 123 and Guiding Principles in the use of Animals in Toxicology. Furthermore, all authors declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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