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Hematological effect of chronic administration of ethanolic extract of *Garcinia conruana* seed on rat

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ABSTRACT

Present work deals with the haemalogical effect of ethanolic extract of *Garcinia conruana* seed on wistar albino rats. 20 rats weighing 150-200g were divided into four groups of five rats per group, and were gavaged with the extract of *Garcinia conruana* seed daily for 21 days. Group 1 served as the control group and was fed with standard animal feed only, while group 2, 3 and 4 were the test groups. In addition to the standard feed, 100mg/kg, 200mg/kg and 300mg/kg of the extract was orally gavaged to the test groups 2, 3 and 4 respectively. The result showed increase in Hemoglobin concentration (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), Mean Cell Hemoglobin (MCH) and Mean Cell Volume (MCV) but with a significant decrease in Mean Cell Hemoglobin Concentration (MCHC), total White Blood Cell (WBC) and differentials ($P < 0.001$, $n=5$). This extract therefore is noted to have a mild erythropoietic effect but with a moderate leucopenia which is characterized by lymphocytosis but decrease in all other WBC lines.

Keywords: *Garcinia conruana*; Haematological Indices; RBC; WBC.

INTRODUCTION

Garcinia conruana (Family-*Guttifera*) seed is a brown nut-like seed, used in traditional medicine (Braide, et al., 1989), serves as an antidote to the effect of strophantus gratus, as a guinea worm remedy, Poison antidote, treatment of diarrhea, hepatitis, asthma, common cold, cough, dysmenorrhoea and anti-anemia (Holmes, 2001; Lewis, 1977; Dalziel, 1956). The consumption of this seed has increased globally since it's used medically and traditionally. It is ingested in relatively large amount at various social gathering and so a number of studies have been done while pondering over the possible toxicity in humans vis-versa the pharmacotherapeutic effects of this plant product (Ahumibe, et al., 2009).

It has been shown that chronic ingestion causes histological changes in the liver, kidney and gastrointestinal tract of rats (Braide, et al., 1989). Its active phytochemical components have been shown to have a protective action against chemically-induced hemolysis in G6PD deficient human red blood cells, anti-inflammatory and antipyretic activity (Braide, 1990; Braide, 1993). It has

hypoglycaemic (anti-diabetic) activity, bronchodilatation in man and antispasmodic effect on smooth muscle (Iwu, 1985; Iwu, et al., 1990; Braide, et al., 1989). In view of the extensive use of *G. conruana* nut, with little or no documented fact on the possible adverse effect on some physiochemical systems, it therefore becomes mandatory to investigate into the potential effects of chronic administration of ethanolic extract of *Garcinia conruana* on haematological indices in rats and to subsequently evaluate whether its ethnopharmacological uses such as mentioned above could have side effects such as destruction of blood cells which is common with the use of most chemotherapeutic agents.

MATERIALS AND METHODS

Collection and Preparation of plant extract: Seeds of *Garcinia conruana* (*Guttiferae*) were obtained from a local market (Itam Market), Akwa Ibom State, and were authenticated by a Taxonomist of the department of Botany, University of Uyo. The seeds were rinsed with water to remove debris, peeled, air-dried and pulverized by grinding using a manual blender. The total weight of the *Garcinia* seed was 896g. The powder was divided into two (448g each) and put into two different 200ml conical flask containing ethanol (98.90%). The suspension was agitated with an electric blender for about 10 minutes then allowed to store in the refrigerator (4°C). Twenty four (24) hours later, it was filtered using glass funnel with cotton wool. The filtrate was evaporated to dryness by heating in a water bath at 40°C. The extract weight was 57.46g (12.83% yields) of crude extract. This was reconstituted in distilled water to an appropriate concentration, stored in refrigerator awaiting the administration. Lethality studies put the LD50 of *Garcinia conruana* extract at about 450.24mg/kg. From here, convenient doses were chosen to preclude the lethal range.

Collection and maintenance of animals: Twenty adult albino rats of both sexes weighing 150-200g were randomly divided into 4 groups of five rats per group. Group 1 served as the control, and was fed with standard animal feed (Bendel Feed and Flour Mill Ltd, Benin) only, while groups 2, 3 and 4 were the test groups and in addition to the standard animal feed they were also gavages with 100mg/kg, 200mg/kg and 300mg/kg ethanol extract of *Garcinia conruana* respectively.

All animals were weighed before treatment commenced and were housed in a standard wooden cage with wooden shavings as their beddings, kept and maintained in the animal house unit of the department of Pharmacy and Toxicology, Faculty of Pharmacy, University of Uyo. They were allowed free access to water ad libitum, good light and maintained in room temperature. After 21 days of extract administration, all the experimental rats were sacrificed by a blow on the head, dislocating the neck and the whole blood was obtained by Cardiac Puncture into EDTA bottles for blood analysis of some haematological indices. This research was carried out in University of Uyo, according to the rules in Nigeria (Revised Helsinki Declaration, 2008) governing the use of laboratory animals as acceptable internationally.

Determination of Hematological Indices: The determination of the hematological indices was done within two hours of sample collection using SYSMEX (KX 21) Automated Hematologic Analyser in the hematology unit of the University of Uyo Teaching Hospital.

Statistical analysis: This was carried out using windows Statistical Package for Social Sciences (SPSS) version 13. One way analysis of variance was adopted for comparison, and the results were subject to post hoc test using least square deviation

(LSD). The data were expressed as mean \pm standard error. $P < 0.05$ were considered significant.

RESULT

Table 1 and 2 shows chronic administration of *Garcinia conruana* extract for 21 days produced various effects on the hematological parameters of wistar albino rats. There were increases in PCV, Hb, RBC, MCH, MCV and lymphocyte count, while MCHC, total WBC and differentials were decreased. These were significantly different from control at ($P < 0.01$).

Table- 1: Hematological Indices of Rat Treated with Ethanolic Extract of *Garcinia conruana* Seeds.

Group	PCV (%)	Hb (g/dl)	RBC (mm ³)	MCH (pg)	MCV	MCHC
G1 (Control)	43.2 \pm 2.11	13.52 \pm 1.47	6.9 \pm 0.2	17.1 \pm 0.48	62.46 \pm 1.7	27.34 \pm 0.17
G2	49.36 \pm 0.85	14.66 \pm 1.93	7.39 \pm 0.1	17.32 \pm 0.04	66.78 \pm 0.44	26.18 \pm 27**
G3	46.3 \pm 2.06	13.64 \pm 1.95	7.02 \pm 0.3	17.72 \pm 0.29	68 \pm 1.14	26.06 \pm 0.17**
G4	48.47 \pm 1.18	14.30 \pm 1.74	7.34 \pm 0.23	17.48 \pm 0.45	66.48 \pm 0.45	26.03 \pm 0.18*

- *Significantly different from G2 ($P < 0.05$); **Significantly different from G1 ($P < 0.01$)

Table-2: Total and Differential White Blood Cell Count of wistar Albino rats treated with Ethanolic Extract of *Garcinia conruana* Seed.

Group	WBC	Platelets	Lym (%)	N %	E %	M%	B%
G1	13.52 \pm 1.47	1132.6 \pm 2.25	68.72 \pm 1.76	31.28 \pm 1.76	0.52 \pm 0.20	3.02 \pm 0.66	0.56 \pm 0.32
G2	11.66 \pm 1.95	686 \pm 2.45 ^a	79.48 \pm 1.66**	20.52 \pm 1.66**	0.46 \pm 0.34	3.0 \pm 0.76	0.51 \pm 0.22
G3	11.44 \pm 1.38	1033.6 \pm 1.86 ^{a,b}	81.98 \pm 0.99**	18.02 \pm 0.99**	0.38 \pm 0.30	2.76 \pm 0.92	0.48 \pm 0.46
G4	11.3 \pm 1.74	987.2 \pm 1.96 ^{a,b,c}	79.52 \pm 2.9**	20.48 \pm 2.9**	0.48 \pm 0.64	2.98 \pm 0.94	0.50 \pm 0.12

- G1 is control
- **Significantly different from G1 ($P < 0.01$); ^aSignificantly different from G1 ($P < 0.001$); ^bSignificantly different from G2 ($P < 0.001$); ^cSignificantly different from G3 ($P < 0.001$)

DISCUSSION

The mechanism leading to the increase in RBC parameters is probably mediated by the anti-oxidant property of *Garcinia conruana* seed extract which has been variously demonstrated by other researchers. This gives the extract the hemopoietic, protective and stimulating potential. Previous research has shown that, prophylactic and therapeutic oral administration of anti-oxidant supplement significantly increased cells of hemopoietic origin in animals exposed to potentially lethal dose of radiation (Chris, et al., 2008). Similarly, anti-oxidants such as Vit C, Vit E, succinate and alpha-lipoic have been used to abolish various forms of chemically and metabolically induced oxidative stress to which human hemopoietic cell lines is exposed to (Chris, et al., 2008). The direct link between anti-oxidant activity and hemopoietic boosting effect was also demonstrated by the observation that ascorbic acid supplementation, through its action as a free radical scavenger, increased significantly the hemoglobin levels of children suffering from sickle cell anemia (Jaja, et al., 2002). In a similar way, the active phytochemical component of *G. conruana* seed extract has been shown to have a protective action against chemically-induced hemolysis in G6PD deficient human red blood cells (Braide, 1990).

Two teams of researchers at different times and locations have also demonstrated unequivocal increases in RBC parameters in their work on the effect of the *G. conruana* seed extract on rats and rabbits respectively, as was observed in this study (Esomonu, et al., 2005; Uniqwe, et al., 2009). We also observed that while the

erythropoietic modulated hemocyte lines showed increases in response to administration of the extract, the white blood cells lines rather decreased with exception of lymphocytes. This observation suggests that *G. conruana* extract effect relating to increase in RBC lines may be effected through an influence on the stimulant cytokine erythropoietin (Ahumibe, et al., 2009). The selective increase in lymphocytes count in this study was similarly observed by other researchers. Adedeji et al., 2006 in their work with pullet chicks observed that, though no significant increase occurred in other white cell lines, lymphocytes specifically proliferated. Ahumibe et al., 2009 also made similar observation in their study of the effect of the extract on erythrocyte membrane integrity and selected hematological indices in male Albino rats. This probably explains the antimicrobial effect of *G. conrauna* extract, in view of the major role that lymphocytes assume in the immune mechanism of the body in both man and animals.

CONCLUSION

Garcinia conruana seed extract through its anti-oxidant property has hematopoietic, stimulating, enhancing and protective effects. Its use as antianemia, antimicrobial and immune booster is therefore supported.

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