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Haematological Effect of Ethanolic Extract of Earth Ball (Icacinia manni) in Wistar Rats: The Result of Vitamin C Co-Administration

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Abstract **Original Research Article**

Background of study*:** Earth ball (Icacinia manni), a tuberous plant has found its application in the preparation of animal and bird feeds. Unfortunately there are conflicting information on its hematological effects when consumed by animals. So we decided to independently verify this and the possible effect of vitamin C on any haematological derangement since an earlier study suggests its reproductive toxicity could be due to oxidative stress. ***Methodology: Twenty male wistar rats were randomly assigned into four groups of 5 rats each. Group 1 was control, group 2 the low dose Icacinia manni, group 3 the high dose Icacinia manni while group 4 was the high dose Icacinia manni + vitamin C groups. All rats had access to potable water and rat feeds ad libitum. At the end of the treatment period (28 days) animals were anaesthesized and blood collected for evaluation of hematological indices ***Results:*** Our results showed a significantly increased hemoglobin concentration in the high dose group compared with each of control, low dose and high dose + vitamin C groups ($P<0.05$ in each case). Red blood cell count was significantly higher in the high dose extract-treated ($P<0.05$) and high dose extract-treated + vitamin C ($P<0.05$) groups compared with control. Hematocrit level was significantly increased in the high dose group compared with control ($P<0.05$). There were no significant differences in the mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width, platelet count, total white blood cell count, neutrophil count and lymphocytes count. Monocyte count in all Icacinia manni treated groups was significantly decreased ($P<0.05$ each) compared with control. Eosinophil count was significantly decreased in high extract treated group compared with control ($P<0.05$). ***Conclusion*:** In conclusion, extract of high dose Icacinia manni tuber has negative and in other cases positive effects on some hematological indices, but which vitamin C does not influence.

Keywords: Ethanolic extract, Icacinia manni, hematological, vitamin C.

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INTRODUCTION

Icacina manni or as commonly called ‘’earth ball’’ is an all season evergreen shrub found in both tropical and non-tropical religions of the world. The underground part of the plant develops into a well modified root tuber made up of mainly carbohydrate. It is not directly consumed by humans (Agyakwa and Akobunda, 1998). It also contains anti-nutrient factors like hydrogen cyanide, phytic acid, oxalic acid, alkaloids and tannins which limit its use as animal feeds (Antai and Obong, 1992). The phytochemistry of the tuber is affected by various processing methods (Antai and Obong, 1992).

This tuber which is rich in carbohydrate may be consumed indirectly by man. In fact, several studies have been conducted while others are on-going to modify its processing methods so that it could be used as a standard replacement for energy content in animal and bird feeds (Umoren *et al*., 2008, Umoren *et al*., 2007, Effiong *et al*., 2014, Effiong and Jimmy 2020).

Precious studies on various forms of extracts of Icacina manni have shown it to have both positive and negative effects on body functions. Essien and Sam (2018) and Essien (2021) did not observe any hematological alteration following administration of Icacinia manni extract while Solomon *et al*., (2011) observed an increase in total white blood cell count and a low packed cell volume following consumption of Icacinia manni extract. Other known effects of Icacinia manni include increased in dressed weights, lungs, gizzard, liver, neck, shank and intestine (Umoren *et al*., 2007) as well as elevated serum liver enzymes (Udokang *et al*., 2011).

The hematological system is an essential system owing to its nutritive, respiratory, excretory, transport, regulatory and defensive functions (Sembulingam and Sembulingam, 2011).

Vitamin C is an essential nutrient involved in tissue repair and enzymatic production of several substances in the body (Sembulingam and Sembulingam, 2011). It also functions as a potent antioxidant (Fassiet, 1973, Umoren *et al*., 2007). It functions as an enzyme substrate and co-factor and an electron donor in mainly enzymatic reactions that mediate variety of essential biological functions (US National Institute of Health, 2016, Meister, 1994).

Much attention has been paid to the search for an alternative, readily available and inexpensive sources of energy to supplement other energy sources in the poultry and animal feeds due to increasing cost of production of and its consequent economic implications. Icacinia manni is one of those alternative sources been researched upon (Effiong *et al*., 2014, Effiong and Jimmy, 2019, Umoren *et al*., 2007, Umoren *et al*., 2008). Unfortunately studies have presented conflicting reports on the effect of the plant on the hematoogical system. We therefore thought it necessary to do our own independent study on the effect of Icacinia manni on hematologica indices and possible effect following co-administration with an anti-oxidant (Vitamin C) as oxidative stress has been shown to mediate several pathologies or tissue dysfunctions (Aribo *et al*., 2018, Marriero *et al*., 2017, Dhalla *et al*., 2000).

MATERIALS AND METHODS

Ethical approval for Animal Study

Approval for use of animals was obtained from the local Research Ethics Committee of the University of Uyo, Uyo.

Plant collection

Tubers of Icacinia manni were harvested from the bush in Uyo, Akwa Ibom state, Nigeria and taken to Botany Department, University of Uyo, Uyo for identification.

Preparation of plant extract

The tubers so harvested were washed with water to remove sand, cut into pieces and sun-dried. The dried specimens were used to prepare the extract using 80% ethanol at the Department of Pharmacognosy, University of Uyo, Uyo. In summary, after two weeks of sun-drying, the pieces of Icacinia manni were grind to powder and marcerated in 80% ethanol for 72 hours to produce the crude ethanolic extract. The liquid filtrate was concentrated and evaporated to dryness using a rotary evaporator to produce the extract which was stored at 4˚C till used.

Experimental animals

Twenty male wistar rats used for the study were obtained from the Department of Pharmacology and Toxicology, University of Uyo and transferred to the animal house of the Faculty of Basic Medical Sciences, University of Uyo, where they were kept in cages in a well-ventilated section of the animal house.

Determination of LD 50

The median lethal dose (LD 50) of the extract was estimated using Locke's (1983) method and found to be 894.43/kg.

Experimental Protocol

Twenty male Albino Wistar rats were randomly assigned into 4 groups of 5 rats each. Group 1 served as a control. Group 2 received low dose extract (89.44 mg/kg) daily, group 3 received high dose extract (368.33 mg/kg) daily while group 4 received high dose extract + vitamin C at a dose of 2.8mg/kg. Extract and vitamin were given daily by gavaging for twenty eight days. All animals had free access to drinking water and animal feed.

Sample collection

At the end of the 28 days treatment periods, animals were anaesthesized and blood samples collected through cardiac puncture into well labeled EDTA bottles for determination of relevant hematological parameters.

Determination of hematological parameters

This was evaluated using an automated hematological machine (BC 2800 Mindray Auto Hematology Analizer, USA).

Statistical analysis

Data were expressed as mean ± Standard Error of Mean (SEM) and differences between mean values evaluated by analysis of variances (ANOVA), followed by Tukey's Post-hoc for pair wise comparisons. Values of $P<0.05$ were considered statistically significant. Graph Pad Prism 7.0 Software (GraphPad Inc USA) was used for statistical analysis.

RESULTS

Our results showed variations in some hematological parameters in the Icacinia manni-fed especially the high-dose groups compared with control. There was a significant increase in hemoglobin concentration in the high dose group compared with control ($P<0.05$) as in Figure 1. Red blood cell count/concentration was significantly increased in the high dose extract ($P<0.05$) and high dose extract + vitamin C ($P<0.05$) groups compared with control (Fig 2). Hematocrit levels were significantly increased in high dose extract ($P<0.05$) compared with control (Fig 3). There were no significant changes in the mean corpuscular volume (MCV), mean corpuscular

hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) red cell distribution width, platelet count, neutrophil, lymphocytes and total white cell counts (Figs 4-11). Monocytes were significantly lower in all icacinia manni-fed groups (P<0.05 each) compared with control (Fig 12). Eosinophil percentage was significantly lower in the icacinia manni high dose group (P<0.05) compared with control (Fig 13).

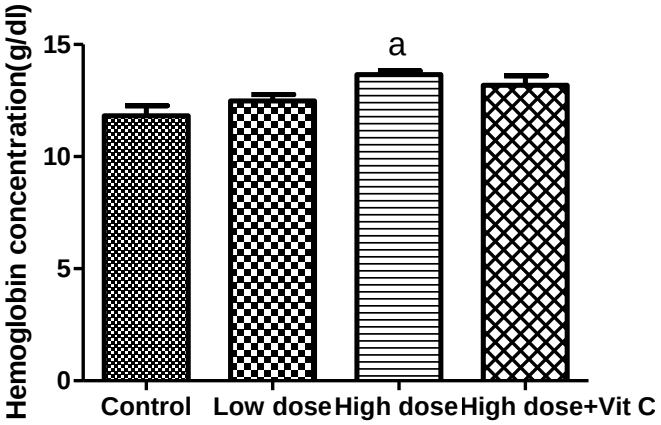

Figure 1: Comparison of hemoglobin concentration in experimental groups. n = 5. [a]p<0.05 vs control. [b]p<0.05 vs low dose group . [c]p<0.05 vs high dose group

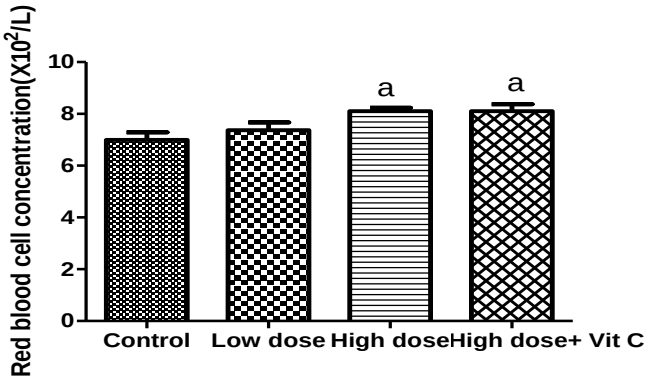

Figure 2: Comparison of red blood cell count in experimental groups. n = 5. [a]p<0.05 vs control group

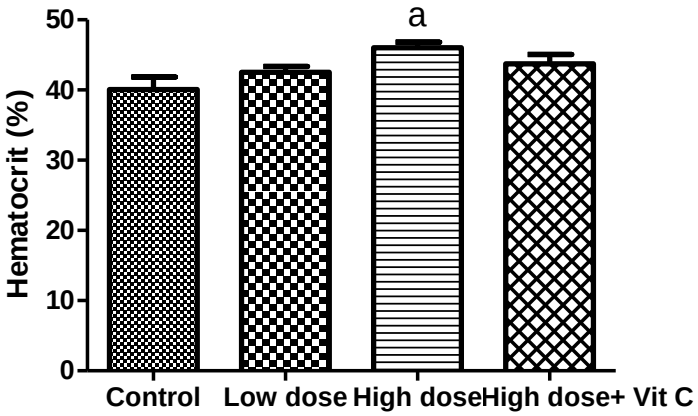

Figure 3: Comparison of hematocrit levels in experimental groups n = 5. [a]p<0.05 vs control group

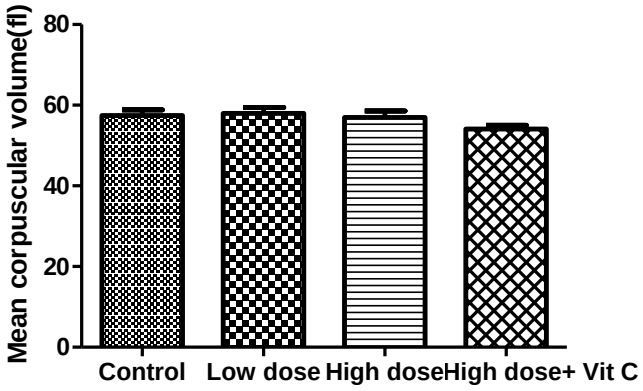

Figure 4: Comparison of mean corpuscular volume in experimental groups. n = 5

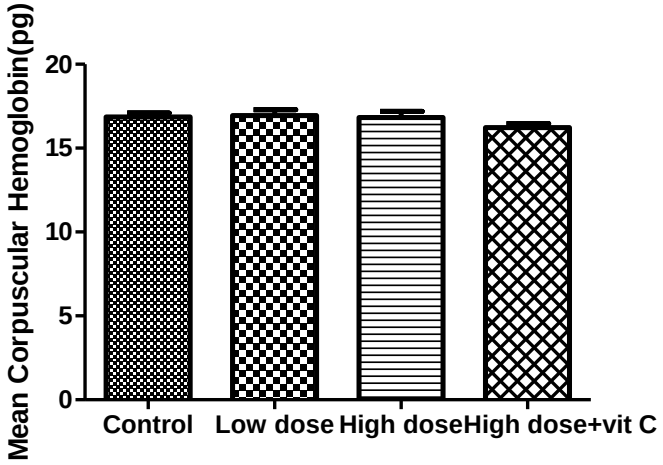

Figure 5: Comparison of mean corpuscular hemoglobin in experimental groups. n = 5

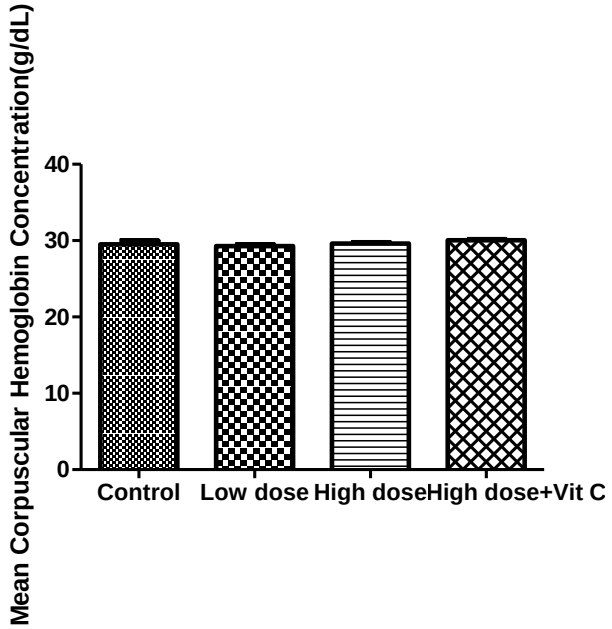

Figure 6: Comparison of mean corpuscular hemoglobin concentration in experimental groups. n = 5

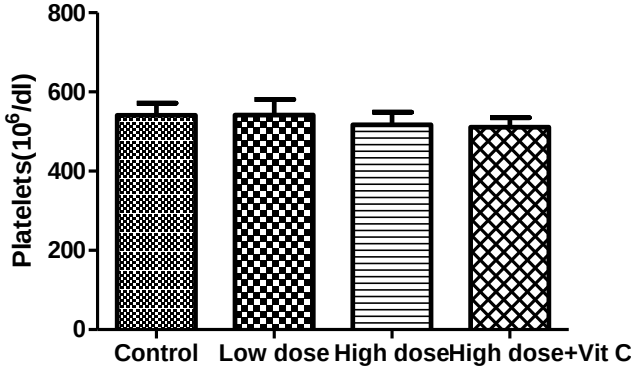

Figure 7: Comparison of platelets count in experimental groups. n = 5

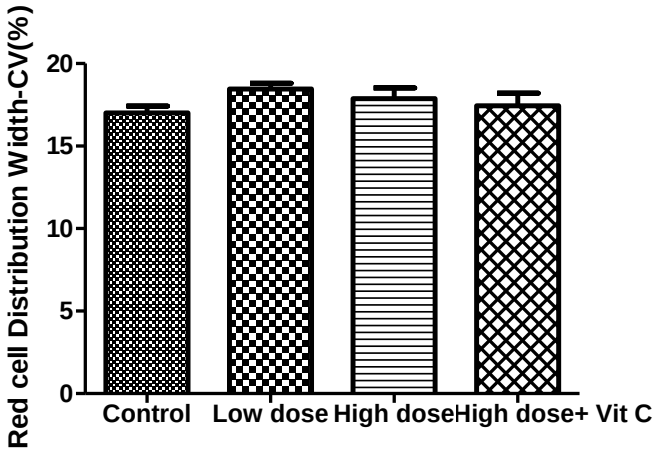

Figure 8: Comparison of red cell distribution width in experimental groups. n = 5

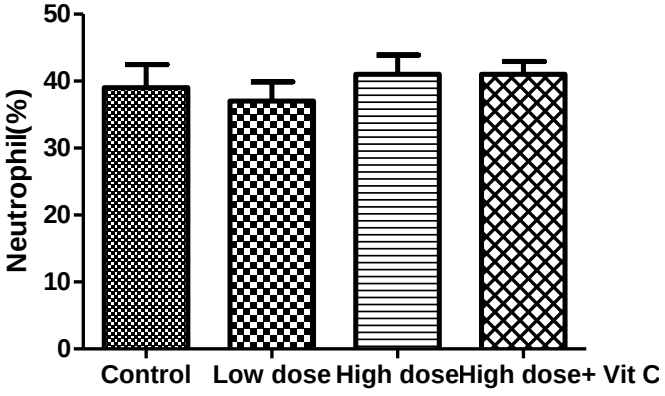

Figure 9: Comparison of neutrophil count in experimental groups. n = 5

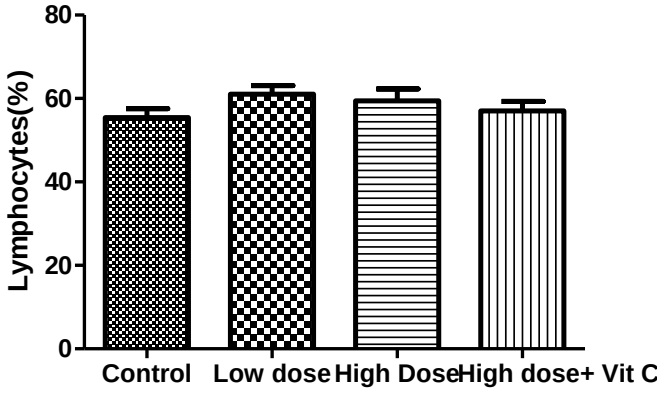

Figure 10: Comparison of lymphocytes count in experimental groups. n = 5

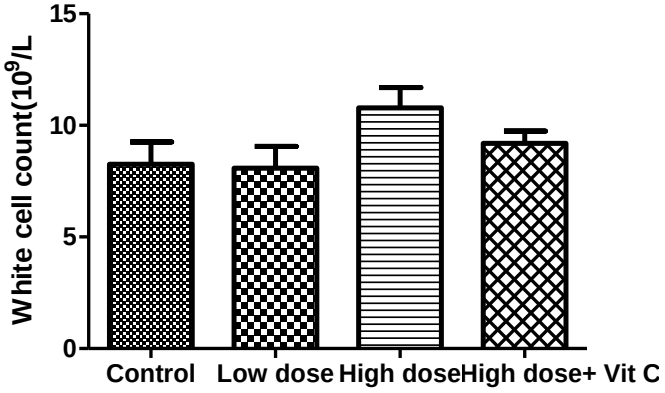

Figure 11: Comparison of total white cell count in experimental groups, n = 5

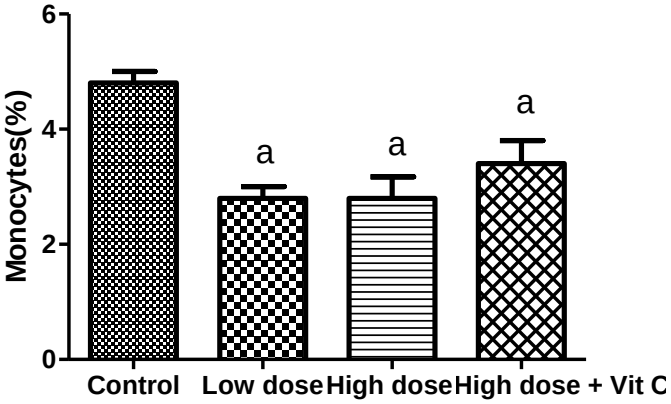

Figure 12: Comparison of monocytes count in experimenatal groups. n = 5. [a]p<0.05 vs control group

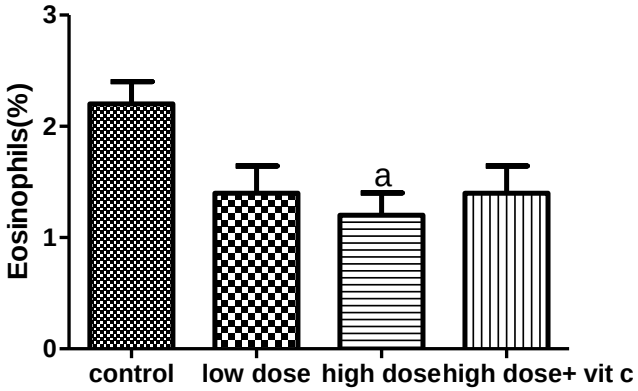

Figure 13: Comparison of eosinophil count in experimental groups. n = 5. [a]p<0.05 vs control

DISCUSSION

The effect of ethanolic extract icaninia manni on hematological parameters in wistar rats was evaluated. The results showed variation in some hematological parameters and its possible explanation is given here.

Our results show a significant increase in hemoglobin concentration in the high dose Icacinia manni extract-administered group compared with control. Red blood cell count and hematocrit were also significantly increased in the high dose Icacinia manni group compared with control. These results are at variance with earlier reports by Essien (2021) who reported that the extract did not have any significant hematological effect. The findings of increases in hemoglobin concentration, hematocrit and red cell count only in the groups treated with high dose extract and not in the low dose extract group suggests that the erythropoietic factors in Icacinia manni and its effect occur in a dose-dependent manner.

Our findings of a dose-dependent significant decrease in the monocyte and eosinophil count in the high dose group compared with control is also contrary to earlier findings by Essien (2021) who found no significant hematological changes associated with Icacinia extract and Solomon *et al*., (2011) who only found an increase in total white blood cell count and not monocytes, eosinophils or other hematological parameters.

The variations in results obtained by our study and those from earlier investigators could have been due to several factors. Firstly, the soils from which the investigators harvested their Icacinia manni tubers were different which might have affected the composition of phytochemicals in their tubers (Inbathamizh and Padmini 2013, Ghasemzadeh *et al*., 2018). Changes in phytochemistry might have affected their actions on body functions. Secondly, phytochemistry of any plant is known to be affected by method of processing the extract or plant, like toasting, fermentation, aqeous, alchoholic, saline, boiling and other methods of processing. Some of these methods could potentiate or enrich while others deplete some plants constituents (Effiong and Jimmy 2019, Antai and Obong, 1992). The alteration in the chemical constituents of these preparations could therefore affect their biological function. The different investigators used different processing methods. For example, Essien (2021) used saline preparation while Solomon *et al*., (2011) used toasted and sundried preparations. We used ethanolic preparation. These factors might have been responsible for variations in biological functions (hematological indices) observed in our study and other investigations.

Vitamin C co-administration with the high dose extract did not produce any significant effect on the hematological indices.

CONCLUSION

We therefore conclude that high dose ethanolic extract of Icacinia manni improves RBC count, hemoglobin concentration and hemoatocrit but reduces monocyte and eosinophil counts in wistar rats.

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