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ORIGINAL ARTICLE

Effects of *Colocasia esculenta* Leaf Extract in Anemic and Normal Wistar Rats

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Background and Objective: *Colocasia esculenta* is a tropical plant primarily grown for its edible starchy corm. It is a food staple in African, Oceanic, and South Indian cultures and is believed to have been one of the earliest cultivated plants. This study was aimed at investigating the hematological effects of *C. esculenta* leaf extract in anemic and normal Wistar rats. **Materials and Methods:** Wistar rats ($n = 30$), aged 2–3 months, weighing 160–220 g, were categorized into five groups (A to E). Groups A and B were orally induced with 1.35 mg/kg bodyweight of methotrexate for anemia. Graded doses of the extract were administered to Groups A to D (A = 300, B = 600, C = 300, and D = 600 mg/kg bodyweight) for 14 days. Group E served as control. Blood samples (3.0 ml) were collected on days 8 and 15 into tri-potassium ethylenediaminetetraacetic acid containers and analyzed using an hematological autoanalyzer (Sysmex KX-21N) following manufacturer's guidelines. **Results:** The acute toxicity test revealed an oral LD_{50} of 6000 mg/kg bodyweight. On day 8, Groups A and B revealed significant decrease ($P < 0.005$) in hemoglobin (Hb), hematocrit (Hct), and red blood cell (RBC) count compared to that of control. On day 15, Group B revealed significant increase ($P < 0.005$) in total white blood cell (TWBC) count; Groups C and D revealed significant increase ($P < 0.005$) in Hb, Hct, RBC, and TWBC compared to that of control. **Conclusions:** This study revealed dose- and time-dependent increase in Hb, Hct, and RBC in normal Wistar rats and leukocytosis in both normal and anemic Wistar rats by crude methanolic extract of *C. esculenta* leaves. These indicate hematopoiesis in normal Wistar rats.

Key words: *Colocasia esculenta*, anemia, hematopoiesis

INTRODUCTION

Anemia is decreased oxygen-binding ability of each hemoglobin (Hb) molecule due to deformity or lack in numerical development as in some other types of Hb molecule.[1] It is also a reduction of Hb and hematocrit (Hct) levels in relation to age, sex, and location of the individual considered. The bone marrow is the major site of hemopoiesis in adult humans.[2] Hematinic drugs stimulate the erythropoietin to produce more blood cells during anemia and this has been demonstrated in medicinal plants.[3] Herbal drugs or medicinal plants have developed a wide spectrum of biological activities.[3]

Colocasia esculenta is also known as cocoyam. It is grown for its edible corms, cormels, leaves, and for other traditional uses by subsistence farmers.[4] It is generally cultivated for enlarged underground starch-rich corms and cormels called tubers. The main problem in the consumption of the leaves of *C. esculenta* is the presence of antinutritional factors. These factors may have adverse effect on health through inhibition of protein digestion, growth, and iron and zinc absorption. However, all parts of the raw *C. esculenta* plant are known to contain oxalate, a toxic compound, which must be destroyed by thorough cooking before eating.

The leaves of *C. esculenta* have been reported to have nutrients, including minerals and vitamins such as calcium, phosphorus, iron, Vitamin C, thiamine, riboflavin, and niacin.[5] The leaves contain mucilage and are an effective nervine tonic. The leaf juice is a stimulant, expectorant, astringent,

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and appetizer. It is used as a folk medicine to cure diarrhea. *C. esculenta* possess hypoglycemic effect due to the presence of cyanoglycoside.[6] Its hypolipidemic activity has been reported due to the presence of arabinogalactan and mono and digalactocyl diacylglycerols.[7]

Despite the numerous medicinal properties and uses of this extract, there is paucity of information on its biochemical and hematological effects in anemic and normal Wistar rats. This study will advance knowledge on the possible hematopoietic and leukocytotic effects of *C. esculenta* leaf extract. The aim of this study was to investigate the biochemical and hematological effects of *C. esculenta* leaf extract in anemic and normal Wistar rats. The specific objectives were to determine (i) the acute toxicity (LD_{50}) of the extract and (ii) the biochemical and hematological parameters of anemic and normal Wistar rats after oral administration of graded doses of the extract.

MATERIALS AND METHODS

Collection and authentication of plant materials

The leaves of *C. esculenta* were obtained from Ajalli town, Orumba North Local Government Area in Anambra State, Nigeria, in August 2016, and authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka, Nigeria. A specimen was deposited in the herbarium for future reference with a voucher number of UNH 379a.

Animal housing

Wistar rats ($n = 30$) were purchased and housed in the animal house of College of Medicine, University of Nigeria Enugu campus, Nigeria. They were allowed to acclimatize for 2 weeks and fed with commercially available rat feed and had access to water and feed *ad libitum*. All the Wistar rats in the study were handled according to the international guidelines for handling experimental animals by the American Physiological Society.

Preparation of the extract

The leaves of *C. esculenta* were shade-dried and grinded into fine powder. Two hundred and seventy grams of the powder was soaked in 2.5 L of methanol (CH_3OH) for 48 h. It was sieved with a muslin cloth and filtered with Whatman No. 1 filter paper. The filtrate was evaporated to dryness. The dried extract yield was scrapped out of the stainless bowl and 10 g of it was dissolved in 100 ml of distilled water to get a concentration of 100 mg/ml for use.

Experimental design

Wistar rats ($n = 30$) weighing 160–220 g and aged 2–3 months were divided into five groups of six rats per group and labeled from A to E. Groups A and B were orally induced for anemia with 1.35 mg/kg bodyweight of methotrexate. After 3 days of anemic induction, Groups A, B, C, and D were orally administered with graded doses of the extract (A = 300, B = 600, C = 300, and D = 600 mg/kg bodyweight) based on the Organization of Economic Cooperation and Development's guidelines on drug dosage[8] for 14 days. Group E served as control. Blood samples (3 ml) were collected from all the rats on days 8 and 15 through the retro-orbital plexus of the median canthus into ethylenediaminetetraacetic acid anticoagulant containers.

Acute toxicity test (median lethal dose, LD_{50})

This was performed on rats according to the procedure described by Lorke, 1983,[9] with little modification. The LD_{50} was performed in two stages. In the first stage, three groups of three rats each were treated with 10, 100, and 1000 mg/kg of the extract and observed for the number of deaths in 24 h. Based on the percentage survival rate, four mice were treated with 1500, 3000, 6000, and 12,000 mg/kg bodyweight of extract in the second stage and the number of deaths in 24 h was recorded. The LD_{50} was calculated as the geometric mean of the highest nonlethal and the lowest lethal doses.

Hematological analysis

Hb, Hct, red blood cell (RBC), mean cell Hb concentration, mean cell Hb, mean cell volume, total white blood cell (TWBC), neutrophil, lymphocyte, monocyte, and eosinophil were analyzed using an hematological autoanalyzer (Sysmex KX-21N) following manufacturer's guideline.

Ethics

The procedures followed in this study were in accordance with the ethical standards of ethics committee on animal experimentation.

Statistical analysis

The data were subjected to descriptive statistics and analyzed using Student's *t*-test and one-way ANOVA. $P < 0.05$ was considered statistically significant.

RESULTS

The acute toxicity test revealed an oral LD_{50} of 6000 mg/kg bodyweight. On day 8, Groups A and B (anemic-induced Wistar rats) revealed significant decrease ($P < 0.05$) in Hb, Hct, and RBC count when compared with the control [Table 1]. Groups C and D (normal Wistar rats) revealed a significant increase ($P < 0.05$) in TWBC count when compared with the control [Table 1]. On day 15, Groups B, C, and D revealed

significant increase ($P < 0.05$) in TWBC count; Groups C and D revealed significant increase ($P < 0.05$) in Hb, Hct, and RBC count compared to control [Table 2]. These findings show the possible hematopoietic and leukocytotic effects of *C. esculenta* leaf extract on anemic-induced and normal Wistar rats.

DISCUSSION

C. esculenta possess anticancer, anti-inflammatory, anti-oxidant, antimicrobial, hypoglycemic, and anthelmintic activities. It also has some neuropharmacological activities such as anti-depressant, sedative, and smooth muscle relaxant activities.[10] The phytochemical constituents of *C. esculenta* leaf extract include alkanoids, flavonoids, saponins, tannins, calcium, oxalate, fibers, minerals (calcium and phosphorus), and starch. The leaves of *C. esculenta* have been reported to be rich in nutrients, including minerals and vitamins such as calcium, phosphorus, iron, Vitamin C, thiamine, riboflavin, and niacin.[10,11] However, all parts of the raw *C. esculenta* plant are known to contain oxalate, a toxic compound, which must be destroyed by thorough cooking before eating.

In this study, the observed high LD_{50} of 6000 mg/kg bodyweight in the *C. esculenta* leaves extract indicates that the extract may probably be nontoxic and will be safe for consumption. On the contrary, Kalariya *et al*.,[12] in their work entitled "Effect of hydroalcoholic extract of leaves of

Table 1: The mean±standard deviation of hematological parameters of anemic, normal, and control Wistar rats on day 8 after oral administration of graded doses of crude methanol extract of *Colocasia esculenta* leaves

Parameters	Group A Anemic/300 mg/kg bodyweight extract	Group B Anemic/600 mg/kg bodyweight extract	Group C Normal/300 mg/kg bodyweight extract	Group D Normal/600 mg/kg bodyweight extract	Group E Control
Hb (g/dL)	8.5±0.8*	9.5±0.2*	11.7±0.2	12.1±0.3	11.5±0.3
Hct (L/L)	0.27±0.03*	0.31±0.01*	0.35±0.01	0.36±0.02	0.35±0.01
RBC ($\times 10^{12}$/L)	2.53±0.31*	2.88±0.09*	3.27±0.2	4.1±0.27	3.87±0.23
MCHC (g/dL)	31.48±1.5	30.65±0.5	33.43±0.8	33.61±0.4	32.86±0.6
MCH (Pg)	33.59±0.4	32.99±0.8	35.78±1.5	29.51±1.2	29.72±0.5
MCV (fl)	106.72±2.3	107.64±1.7	107.03±2.5	87.80±1.4	90.44±1.2
TWBC ($\times 10^{9}$/L)	3.5±0.1	4.2±0.1	4.5±0.2*	4.6±0.2*	3.9±0.4
Neutrophil (%)	48±4	47±3	48±1	48±3	51±2
Lymphocyte (%)	50±4	50±3	49±1	49±3	45±2
Monocyte (%)	1±0.5	1±0.5	2±1	2±0.5	2±0.5
Eosinophil (%)	1±0.5	2±0.5	1±0.5	1±0.5	2±0.5

*P<0.05 (significant). Hb=Hemoglobin; Hct=Hematocrit; RBC=Red blood cell; MCHC=Mean cell hemoglobin concentration; MCH=Mean cell hemoglobin; MCV=Mean cell volume; TWBC=Total white blood cell

Table 2: Mean±standard deviation of hematological parameters of anemic, normal, and control Wistar rats on day 15 after oral administration of crude extract of *Colocasia esculenta*

Parameters	Group A Anemic/300 mg/kg bodyweight extract	Group B Anemic/600 mg/kg bodyweight extract	Group C Normal/300 mg/kg bodyweight extract	Group D Normal/600 mg/kg bodyweight extract	Group E Control
Hb (g/dL)	10.5±0.3	10.8±0.4	12.9±0.3*	13.3±0.3*	11.5±0.3
Hct (L/L)	0.35±0.02	0.37±0.02	0.38±0.01*	0.39±0.02*	0.36±0.01
RBC ($\times 10^{12}$/L)	3.7±0.4	4.13±0.22	4.2±0.2*	4.97±0.19*	3.87±0.23
MCHC (g/dL)	30.00±0.6	29.19±0.3	34.86±0.8	34.10±1.2	31.94±0.7
MCH (Pg)	28.38±0.4	26.15±0.8	30.71±1.3	26.76±0.5	29.72±0.6
MCV (fl)	94.59±1.5	89.59±1.2	88.09±0.9	78.47±0.4	93.02±1.3
TWBC ($\times 10^{9}$/L)	4.4±0.2	4.9±0.3*	5.2±0.3*	5.7±0.3*	3.9±0.4
Neutrophil (%)	49±2	46±2	47±2	50±3	51±2
Lymphocyte (%)	48±2	51±2	50±2	48±2	45±2
Monocyte (%)	2±0.5	1±0.5	2±0.5	1±0.4	2±0.5
Eosinophil (%)	1±0.4	2±0.5	1±0.5	1±0.4	2±0.5

*P<0.05 (significant). Hb=Hemoglobin; Hct=Hematocrit; RBC=Red blood cell; MCHC=Mean cell hemoglobin concentration; MCH=Mean cell hemoglobin; MCV=Mean cell volume; TWBC=Total white blood cell

Colocasia esculenta on marble-burying behavior in mice: Implications for obsessive-compulsive disorder," recorded an intraperitoneal LD_{50} value of 1000 mg/kg.

The observed decrease in Hb, Hct, and RBC count on day 8 in the anemic groups [Table 1] could be due to the effects of methotrexate which was used to induce anemia. The anemia which was induced by the methotrexate was not addressed by the extract at this stage of extract administration probably due to short duration which may not be enough to reveal significant effects. The observed leukocytosis in the normal groups on day 8 indicates that the extract may have stimulatory effect on the immune system that resulted in the production of more leukocytes. However, on day 15, the observed increase in Hb, Hct, and RBC count in the normal groups and leukocytosis in the higher extract dose anemic group and the normal groups [Table 2] indicate hematopoietic potentials of the extract. The extract may have stimulated the kidney to synthesize erythropoietin for hematopoiesis to occur.[13,14] The observed effects were dose- and time-dependent since it was noticed at higher dose of the extract and at longer duration of administration. The effects may also be attributed to the diminishing effects of methotrexate at longer duration of extract administration. The observed leukocytosis indicates immune stimulatory potential of the extract.[15] The antinutritional factors present in the extract may also contribute to the observed effects. This also shows that the extract is antigenic by stimulating the defense mechanism for the production of more leukocytes as antibodies to guard against foreign bodies like the antinutritional factor content of the extract. These effects may equally be attributed to some of the phytochemical constituents of the extract such as flavonoid which is a strong antioxidant, protein, saponin, and vitamin.[16] Some of these phytochemicals may support cell growth and suppress apoptosis.[9]

CONCLUSIONS

This study revealed dose- and time-dependent increase in Hb, Hct, RBC, and leukocytosis in normal Wistar rats and leukocytosis in anemic Wistar rats (higher extract dose). The findings suggested that this extract may possess hematopoietic and leukocytotic potentials.

Future recommendation

Further study is recommended to fractionate *C. esculenta* leaf extract using column chromatography and gas chromatography-mass spectrometry to pinpoint the actual components of the extract responsible for the observed effects.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Babushok DV, Li Y, Roth JJ, Perdigones N, Cockroft JD, Biegel JA, *et al*. Common polymorphic deletion of glutathione S-transferase theta predisposes to acquired aplastic anemia: Independent cohort and meta-analysis of 609 patients. Am J Hematol 2013;88:862-7.
2. Liang R, Ghaffari S. Advances in understanding the mechanisms of erythropoiesis in homeostasis and disease. Br J Haematol 2016;174:661-73.
3. Ufelle SA, Ukaejiofo EO, Achukwu PU, Ghasi S. Haemopoietic potentials of crude methanolic extract of *Apium graveolens* leaves in Wistar rats. J Int Res Med Pharm Sci 2015;6:191-201.
4. Abuajah CI, Ogbonna AC, Osuji CM. Functional components and medicinal properties of food: A review. J Food Sci Technol 2015;52:2522-9.
5. Dhanraj N, Kadam M, Patil K, Mane V. Phytochemical screening and antibacterial activity of Western region wild leaf *Colocasia esculenta*. Int J Biol Sci 2013;2:18-23.
6. Vasant OK, Vijay BG, Virbhadrappa SR, Dilip NT, Ramahari MV, Laxamanrao BS, *et al*. Antihypertensive and diuretic effects of the aqueous extract of *Colocasia esculenta* Linn. leaves in experimental paradigms. Iran J Pharm Res 2012;11:621-34.
7. Subhash C, Sarla S, Jaybardhan S. Phytochemical screening of Garhwal Himalaya wild edible tuber *Colocasia esculenta*. Int Res J Pharm 2012;3:181-6.
8. Guidance Document on using Cytotoxicity Tests to Estimate Starting Doses for Acute Oral Systemic Toxicity Tests (OECD Test Guideline No 129); 2010.
9. Ukaejiofo EO, Ufelle SA, Ghasi S, Achukwu PU, Udeani TK, Neboh EE. Myelo-protective activity of fractions of *Vitex doniana* sweet leaves extract in Wistar rats. Med Sci 2015;18:1-10.
10. Lee S, Wee W, Yong J, Syamsumir D. Antimicrobial, antioxidant, anticancer property and chemical composition of different parts (corm, stem and leave) of *Colocasia esculenta* extract. Ann Univ Mariae Curie Sklodowska Sect DDD 2011;24:9-16.
11. Alcantara RM, Hurtada WA, Dizon EI. The nutritional value and phytochemical components of taro [*Colocasia esculenta* (L.) Schott] powder and its selected processed foods. J Nutr Food Sci 2013;3:207.

12. Kalariya M, Prajapati R, Parmar SK, Sheth N. Effect of hydroalcoholic extract of leaves of *Colocasia esculenta* on marble-burying behavior in mice: Implications for obsessive-compulsive disorder. Pharm Biol 2015;53:1239-42.
13. Jelkmann W. Regulation of erythropoietin production. J Physiol 2011;589:1251-8.
14. Pereira PR, Joab TS, Mauricio V, Gerlinde AP. Crude extract from taro (*Colocasia esculenta*) as a natural source of bioactive proteins able to stimulate haematopoietic cells in two murine models. J Funct Foods 2015;18:333-43.
15. Kumar S, Gupta P, Sharma S, Kumar D. A review on immunostimulatory plants. Zhong Xi Yi Jie He Xue Bao 2011;9:117-28.
16. Onuh SN, Ukaejiofo EO, Achukwu PU, Ufelle SA, Okwuosa CN, Chukwuka CJ. Haemopoietic activity and effect of crude fruit extract of *Phoenix dactylifera* on peripheral blood parameters. Int J Biol Med Res 2012;3:1720-3.