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# Prophylactic Induction: Effect of Methanolic Extract of *Synsepalum dulcificum* Leaves on Haematological Profile of Matured Male Albino Rats in Short-Term Lead Exposure

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**Abstract:** The present study was designed to evaluate the possible effects of methanolic extract of *Synsepalum dulcificum* on hematological parameters such as; Hemoglobin (Hb), packed cell volume (PCV), red blood cell (RBC) count, white blood cell (WBC), monocytes, eosinophils, neutrophils and lymphocytes in matured male albino rats in a short-term lead exposure. Twenty (20) matured male albino rats were randomly divided into control group (negative) and treatment groups 1 (positive control), 2 (50 mg/kg), 3(100 mg/kg) and 4 (150 mg/kg). The subjects were treated for fourteen (14) days i.e. seven days treatment with different doses of the methanolic extract of *Synsepalum dulcificum* (SD) and another seven days treatment by gavage with 2.5 $mg.ml^{-1}$ of lead acetate. At the end of the administration window, significant ($p > 0.05$) decrease was noted in the packed cell volume (PCV) of the group that received 2.5 $mg.ml^{-1}$ of lead acetate and 150 mg/kg of *Synsepalum dulcificum* and in the lymphocytes of the control group (negative) compared to the other treated groups while little or no significant ($p < 0.05$) difference was observed in other haematological parameters in the control group when compared to the treated groups in the study. The results therefore shows that Wonder plant, even at higher dose, it is not potent enough in supressing the activities of lead, a heavy metal in the system viewing it from the angle of haematology.

**Key words:** Wonder Plant · Methanolic · Haematology · Lead Acetate · Prophylactic

## INTRODUCTION

*Synsepalum dulcificum* also called miracle berry or wonder plant is a miraculin-rich plant commonly found in the Tropical West Africa which also grows naturally in farms and bushes with short berry fruits that are bright-red when ripe [1,2]. Little or no much works has been done on the plant but most adventures have been into the sweetening property of the plant, which is due to the presence of miraculin, a glycoprotein, in the pulp of the berry and also the phytochemical screening on the plant [3]. The plant have gained relevant applications among African folks for various purposes such as; sweetening foods and drinks for centuries but recently gaining relevant interest globally in food and pharmaceutical industries for vital purposes [4, 5].

As a class of heavy metals, lead is detectable practically in all spheres of ecosystem, it's been concisely studied in various ways and how it affects the haematological indices as well enhances the formation of challenging number of health issues. It is believed to covalently interact with tertiary phosphate ions in nucleic acids and proteins with the ability to impair immune system and renal system function, hepatic lesions, endocrine impairment and cell membrane lipids in cells of the central nervous system [6-9]. Many of these health impacts have been shown to occur even at an increasingly lower exposure which suggests that, there is no safe threshold to lead exposure [10].

The most abundant cell in the blood is the erythrocyte or RBC which has a buffering capacity on the blood and packed with the oxygen carrying proteins, Hb.

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The approximate concentration of erythrocytes in blood is found to be 3.9-5.5 million per µl in women and 4.1-6 million per µl in men [11]. Human RBCs survive in the circulation for about 120 days and worn out blood cells are removed from the circulation by macrophages of the spleen and bone marrows. The signal for removal seems to be the appearance of defective complex oligosaccharides attached to integral membrane protein of the plasmalemma [12].

Leukocytes (WBCs) migrate to the tissue, where they perform multiple functions and most die by apoptosis. Leukocytes are involved in the cellular and humoral defense of the organism against foreign material. The number of leukocytes in the blood varies according to age, sex and physiological conditions. In normal adults they are roughly 6,000-10,000 Leukocytes per µl of blood [11, 13]. Platelets count range from 200,000 to 400,000 per µl of blood and it originates from the fragmentation of giant polypoid megakaryocytes that reside in the bone marrow with a life span of about 10 days and it functions majorly in the prevention of haemorrhage. This work was designed to determine the effect(s) of methanolic extract of *Synsepalum dulcificum* on haematological parameters in short-term lead exposed albino rats.

## MATERIALS AND METHODS

**Extraction of Plant Material:** *Synsepalum dulcificum* fresh leaves were collected at Iseyin in Oyo State, Nigeria. The plant was identified and authenticated by Mr Donatus, a plant scientist in the Department of Botany, University of Ibadan, Oyo State, Nigeria. The fresh leaves of the plant were air-dried, pulverized and extracted exhaustively in methanol. The filtrate was concentrated and evaporated to dryness at 50°C, using rotary evaporator (Stuart Barloworld, Model RE 300). The yield was calculated and the dry extract was stored in a refrigerator at -4°C until it's used for the experiments.

$$\% \text{ yield} = \frac{\text{Extract abtained in mg}}{\text{Crude plant sample in mg}} \times 100$$

**Animal Treatment:** A total number of twenty albino rats weighing between 100-190 g were used in this study. The animals were obtained from the animal house of the Department of Chemical Sciences, Afe Babalola University, Ado-Ekiti, Nigeria. The animals were randomly distributed into cages and acclimatized for two weeks in a well-ventilated room at a room temperature of 28.0 ± 2.0°C under natural lighting condition. The animals were allowed free access to standard feed (Top feeds Ltd., Sapele, Nigeria) and distilled water *ad libitum*. All animals used in this study were handled in accordance with the international, national and institutional guidelines for care and use of laboratory animals as promulgated by the Canadian Council of Animal Care (2009).

**Animal Grouping:** Animals were randomly divided into four groups 1, 2, 3, 4 and the control groups, respectively.

Group 1 (Positive control) received distilled water daily for the first seven days and single daily dose of 2.5 $mg.ml^{-1}$ lead acetate dissolved in distilled water for the last 7days.

Group 2 received single daily dose of 50 $mg.kg^{-1}$ of methanolic extract of *Synsepalum dulcificum* for the first 7 days and single daily dose of 2.5 $mg.ml^{-1}$ lead acetate for the last 7 days.

Group 3 received single daily dose of 100 mg $kg^{-1}$ of methanolic extract of *Synsepalum dulcificum* for the first 7 days and single daily dose of 2.5 $mg.ml^{-1}$ lead acetate for the last 7 days.

Group 4 received single daily dose of 150 $mg.kg^{-1}$ of methanolic extract of *Synsepalum dulcificum* for the first 7 days and single daily dose of 2.5 $mg.ml^{-1}$ lead acetate for the last 7 days.

Control group (Negative control) containing four animals, received only distilled water daily for 14 days. *Synsepalum dulcificum* was administered orally using a calibrated 5 ml syringe with attached polythene cannula.

**Haematological Assays:** The animals were sacrificed by cervical dislocation and dissected. Then, the sample blood collected through the ventricular puncture of the heart at the end of the treatment period. Whole blood was collected from the animals into EDTA bottle and assayed for PCV (%), Hb (g/dl), WBC ($x10^3/mm^3$), RBC ($x10^3/mm^3$), Neutrophils (%), Lymphocytes (%), Monocytes (%), Platelets ($x10^3/mm^3$) and Eosinophils (%) using standard techniques.

**Statistical Analysis:** Data were expressed as Mean ± SE of mean. Comparisons between control values and values of treated groups of albino rats were performed with one-way analysis of variance (ANOVA). Statistical significance was set at $p < 0.05$.

Table 1: The results of the effects methanolic extracts of *Synsepalum dulcificum* on the haematological parameters in short-term lead exposure

| Parameters | Group 1 (Positive control) | Group 2 (50 mg/kg) | Group 3 (100 mg/kg) | Group 4 (150 mg/kg) | Control Group (Negative control) |
|---|---|---|---|---|---|
| PCV (%) | $44.50 \pm 1.50^{b}$ | $40.25 \pm 1.03^{ab}$ | $45.00 \pm 0.82^{b}$ | $34.50 \pm 3.97^{a}$ | $45.25 \pm 1.11^{b}$ |
| Hb (g/dl) | $16.50 \pm 0.28^{b}$ | $15.00 \pm 0.70^{ab}$ | $16.50 \pm 0.29^{b}$ | $14.25 \pm 1.50^{b}$ | $16.50 \pm 0.29^{b}$ |
| RBC $x10^3/mm^3$ | $5.25 \pm 0.10^{b}$ | $4.87 \pm 0.28^{b}$ | $5.40 \pm 0.08^{b}$ | $4.55 \pm 0.32^{a}$ | $5.35 \pm 0.18^{b}$ |
| WBC ($x10^3$ $mm^3$) | $4.80 \pm 0.21^{a}$ | $4.85 \pm 0.21^{a}$ | $5.30 \pm 0.33^{a}$ | $4.65 \pm 0.170^{a}$ | $4.97 \pm 0.49^{a}$ |
| Neutrophils (%) | $47.75 \pm 1.03^{a}$ | $50.50 \pm 1.50^{a}$ | $49.25 \pm 0.47^{a}$ | $48.50 \pm 0.50^{a}$ | $46.75 \pm 2.06^{a}$ |
| Lymphocytes (%) | $45.50 \pm 2.36^{bc}$ | $40.00 \pm 0.81^{ab}$ | $40.25 \pm 0.25^{ab}$ | $39.50 \pm 0.95^{a}$ | $46.75 \pm 2.93^{c}$ |
| Monocytes (%) | $6.25 \pm 1.44^{a}$ | $6.00 \pm 1.15^{a}$ | $7.00 \pm 1.29^{a}$ | $7.00 \pm 0.58^{a}$ | $6.00 \pm 0.71^{a}$ |
| Platelets ($x10^3/mm^3$ | $2.00 \pm 40.16^{a}$ | $2.15 \pm 0.30^{a}$ | $2.11 \pm 0.28^{a}$ | $2.20 \pm 0.27^{a}$ | $1.80 \pm 0.08^{a}$ |
| Eosinophils (%) | $3.00 \pm 0.71^{ab}$ | $3.50 \pm 0.96^{ab}$ | $2.50 \pm 0.91^{a}$ | $5.00 \pm 0.57^{b}$ | $2.75 \pm 0.75^{ab}$ |

Means values within a row with S.E and different superscripts a, b, c which shows significant differences, ($p = 0.05$ is considered significant).

## RESULTS

**Haematological Parameters:** The results of the prophylactic effects of different doses of methanolic extract of *Synsepalum dulcificum* on the hematological parameters of albino rats in short-term lead exposure are shown in the table 1. No mortality was observed at any given concentrations of the lead as studied during the experiments. The result was noted in terms of significant differences ($p < 0.05$) in the level of blood parameter in the treatment groups and the control groups after the treatment window.

## DISCUSSION

The current study was designed to examine the effects of the *Synsepalum dulcificum* leaves extract on the haematological profile of the tested animals exposed to lead. It has been shown in several studies that some of the haematological parameters exhibit considerable variations at different periods of life and disease conditions and this could be used for clinical pathological diagnosis and further studies [14-16]. Also, studies have clearly shown that aside from being a cell-target model for cellular lead toxicity in non-excitable cells, erythrocytes are the most important means of conveying lead throughout the body in human and this heavy metal has been well studied to be using the same transport means in human [17].

From the present study, haematological profile in the animals were found as follows, no significant ($p < 0.05$) difference was observed in the PCV of the Control group (negative) [45.25 ± 1.11%] and that of Control group (positive) [44.50 ± 1.50%] compared to that of the treatment groups which were group 2 [40.25 ± 1.03%] and group 3 [45.00 ± 0.82%], but group 4 recorded 34.50 ± 3.97% that took the highest dose of *Synsepalum dulcificum* showed significant ($p > 0.05$) difference from the other groups compared to that of the control groups, this suggests that the plant could probably reduces haematocrit at higher dose. In the same vein, the Hb levels showed significant ($p < 0.05$) difference as indicated across the groups; control group (negative) [16.50 ± 0.29 g/dl], control group (positive) [16.50 ± 0.28 g/dl] and group 3 [16.50 ± 0.29 g/dl], but significant decrease was observed both in the group 2 and group 4 (showing the least value). Also, the RBCs ($x10^3/mm^3$) mean value was observed to have been significantly reduced in the control group (positive) with mean value of 5.25 ± 0.10 ($x10^3/mm^3$) when compared to that of the control group (negative) and subsequently decreased in the tested groups as well. It is possible that the decrease observed in RBCs ($x10^3/mm^3$) may be due haemolysis and damage to hematopoietic tissues by lead [18].

The WBC of the control group (negative) was observed to have a mean value 4.80 ± 0.21($x10^3$ /$mm^3$) which differed significantly ($p < 0.05$) from that of the control group (positive) [4.97 ± 0.49 ($x10^3$ /$mm^3$)] and the other treated groups but significantly ($p > 0.05$) difference from that of group 4 with the highest dose (150 mg/kg), this shows that the heavy metal could probably had affected the production of WBC in the animals which took lead acetate alone. Significant increase was observed in the level of WBC concerning the group that was administered with 100 mg/kg; this could probably indicate that the plant reinforced the defence system of the animals [20]. The neutrophils count percentage of the control group (positive) has a mean value of 47.75 ± 1.03% which is significantly decreased ($p < 0.05$) compared to that of control group (negative) [46.75 ± 2.06%]. Also, in the treated groups the values decreased significantly ($p < 0.05$) in concentration dependent manner. Moreover, lymphocytes percentage of the control group (positive) with mean value of 45.50 ± 2.36% was found to be significantly ($p < 0.05$) decreased compared to 46.75 ± 2.93% observed in the control group (negative) and

significantly ($p < 0.05$) different from the other treated groups after the treatment window.

From the table 1, the monocytes percentage of the control group (positive) was significantly ($p > 0.05$) increased with mean value of 6.25 ± 1.44% compared to that of control group (negative) [6.00 ± 0.71%], while significant ($p > 0.05$) increase also was observed in the treated groups when compared to that of control groups. Meanwhile, significant ($p < 0.05$) difference was observed in the platelets (x103/mm$^3$) mean values in all the groups when compared to that of control (negative). Concerning the eosinophils percentage, significant ($p < 0.05$) decrease was indicated in the control group (negative) when compared to control group (positive) and significant ($p > 0.05$) increase was observed in the group 4 [5.00 ± 0.57%], that received the highest dose (150 mg/kg) when compared to the other treated groups and the control groups.

## CONCLUSION

In the study, it has been shown that *Synsepalum dulcificum*, wonder plant is not potent enough in supressing the activities of lead, a heavy metal in the system viewing it from the angle of haematology and also reducing hematopoietic system synergistically with the activity of the heavy metal used. The plant at higher dosage showed little or no effect on the activity of lead in the haematological parameter.

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