

EFFECT OF METHANOLIC EXTRACT OF *Hibiscus sabdariffa* ON SOME HEMATOLOGICAL PARAMETERS IN LEVODOPA-INDUCED ANEMIA

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ABSTRACT

The effect of oral administration of methanolic extract of *Hibiscus sabdariffa* on body weight, red blood cell count (RBC), packed cell volume (PCV), hemoglobin (Hb), hematocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration of albino wistar rats were studied. Sixteen albino rats were grouped randomly into four groups (n = 4). Control group: (Group A) was given distilled water and normal rat chow. Experimental Group B received 0.4 ml of levodopa only. Experimental Group C received 0.4 ml of levodopa plus 300 mg/kg of *Hibiscus sabdariffa* extract. Experimental Group D received 0.4 ml of levodopa plus 600 mg/kg of *Hibiscus sabdariffa* extract. At the end of 4 weeks of study, results showed a decrease in body weight of group C and D. The level of PCV was significantly reduced in Group B, C and D that received levodopa compared to PCV levels before administration. Group C and D showed significant increase ($P < 0.05$) in PCV, RBC, Hb, Hct, MCV, MCH, and MCHC levels when compared to group B in a dose-dependent manner. However, there was no significant increase ($P > 0.05$) in PCV, RBC, Hb, Hct, MCV, MCH, and MCHC levels between group C and D respectively when compared with group A (Control). The study suggests that *Hibiscus sabdariffa* increases some hematological parameters in levodopa-induced anemic rats and might be beneficial in the treatment of anemia.

Keywords: *Hibiscus sabdariffa*, Packed Cell Volume, Red Blood Cell Count, Hematocrit, Hemoglobin, Mean Corpuscular Volume, Mean Corpuscular Hemoglobin, Mean Corpuscular Hemoglobin Concentration, Levodopa, Anemia.

INTRODUCTION

Roselle (*Hibiscus sabdariffa*) locally known as "Yakuwa" is a well-adapted crop in the semi-arid zone of West Africa including Nigeria and it is generally planted

as a border crop. The leaves are used as vegetable and the floral parts in the preparation of "Sobo," a local drink. The stem provides fiber and the seeds are eaten by scavenging poultry (Philips, 1977). Roselle calyx has been shown to contain phytic acid, tannin and glycosides such as delphinidin-3-monoglucosides and delphinidin which are toxic to animal and human tissue at high concentrations (Morton, 1987; Ojokoh *et al.*, 2002). Extracts are used in folk medicine in the treatment of variety of ailments including high blood pressure, liver diseases and fever (Dalziel, 1973; Wang *et al.*, 2000; Ross, 2003). The use of blood examination as a way of assessing the healthy status of animals have been documented (Muhammad *et al.*, 2000). Experimental model in mice has shown induction of autoimmune hemolytic anemia with levodopa (Linstrom *et al.*, 1977; Sharon and Naor, 1989). Therefore, this work is aimed to evaluate the effect of methanolic extract of *Hibiscus sabdariffa* on some hematological parameters in levodopa-induced anemia in rats.

MATERIALS AND METHODS

Sixteen albino rats (200 - 230 g) were obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria, Nsukka and maintained in a temperature of 25°C - 30°C, 12 hours light and 12 hours dark cycle (approved institutional guidelines that are in compliance with international laws and policies). All animals were given free access to tap water and regular pelleted rat chow during the experiments. During the experiment the animals were weighed and randomly selected into four experimental groups (n=4). Control group: (Group A) was given distilled water and normal rat chow. Experimental Group B received 0.4 ml of levodopa only. Experimental Group C received 0.4 ml of levodopa plus 300 mg/kg of *Hibiscus sabdariffa* extract. Experimental Group D received 0.4 ml of levodopa plus 600 mg/kg of *Hibiscus sabdariffa* extract.

Acute Toxicity Test

The LD₅₀ on *Hibiscus sabdariffa* was carried out employing the method by Lorks (2000). This test involved a total of ten rats and carried out in 2 stages. Animals received oral administration of methanolic extract of *Hibiscus sabdariffa*.

Stage 1 involved six rats. They were grouped into 3 groups (n=2). Group 1 received 10mg/kg of methanolic extract of *Hibiscus sabdariffa*. Group 2 received 100 mg/kg of methanolic extract of *Hibiscus sabdariffa*. Group 3 received 1000 mg/kg of methanolic extract of *Hibiscus sabdariffa*. The animals

were constantly monitored for a period of 5 hours, then the next 6 hours and then over a period of 24 hours. No death was recorded.

Stage 2 was grouped into 4 (n=1). Group 1 received 1500 mg/kg, Group 2 received 2500 mg/kg, Group 3 received 3500 mg/kg, and Group 4 received 5000 mg/kg of methanolic extract of *Hibiscus sabdariffa* respectively. All animals were monitored again over a period of 24 hours. No death was also recorded.

Extract Preparation

Fresh calyces of *Hibiscus sabdariffa* were bought from Elele market in Rivers State, Nigeria. They were sorted to remove debris and dust particles. Large quantities of the calyces were then collected and sun dried for seven days. After drying, they were milled with a mortar and pestle to get a coarse powder used for the extraction. About 200g of the grounded form was soaked in 1000 ml of methanol and placed in a mechanical shaker for 48 hours before filtering with a white handkerchief into a clean bottle. The filtrate was then concentrated to dryness at 50^oC in an electric oven (gallenkamp). It produced a semi-solid mass when dried and stored in an air tight container in the refrigerator below 10^oC. 1.1 g of the extract is then measured using an electric weighing balance and then dissolved into 11 ml of distilled water to give a stock solution of 100mg/ml. The extract was administered orally using a 2 ml syringe without needle. This was done carefully to prevent damage of the alimentary canal of the mice.

Sample Collection

Packed cell volume (PCV) was measured weekly for four weeks. At the end of four weeks experiment, animals were sacrificed; 5 ml of blood was obtained by cardiac puncture under chloroform anesthesia for RBC, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) analysis.

Statistical Analysis

Results are expressed as mean \pm SEM. Statistical significance of the differences observed between control and experimental groups (ANOVA) was evaluated by Turkey's multiple comparison at $P < 0.05$.

RESULTS

Results in figure 1 showed statistically significant decrease in the final body weight of Group C and D rats respectively (147.20 ± 5.8 and 142.00 ± 15.62) at $P < 0.05$ compared to Control group A (220.80 ± 3.28). There was also statistically significant decrease in final body weight of Group 2 and 3 compared to Group B rats (167.60 ± 10.49) at $P < 0.05$. Figure 2 showed the level of PCV before and after administration of levodopa. As expected, the level of PCV was significantly reduced in Group B, C and D that received levodopa compared to PCV levels before administration. Figure 3 showed weekly administration of methanolic extract of *Hibiscus sabdariffa* significantly increased PCV levels in Group C and D rats (45.25 ± 1.65 and 42.50 ± 1.76) at $P < 0.05$ compared to Group B rats (24.25 ± 1.84). However, there was no statistically significant difference between Group C and D compared with Control (46.00 ± 1.29) at $P > 0.05$. Figure 4 showed statistically significant difference in RBC between Group D (8.49 ± 0.37) compared Control (7.36 ± 0.42) at $P < 0.05$. There was no statistically significant difference in RBC between Group C (6.68 ± 0.54) compared with Control at $P > 0.05$. Statistically significant difference occurred in RBC between Group B (3.74 ± 0.12) compared to Control, Group C and D respectively at $P < 0.05$. There was statistically significant difference in hemoglobin and hematocrit between Group B (5.00 ± 0.22 ; 25.47 ± 1.64) compared to Control (10.88 ± 0.30 ; 40.62 ± 1.94), Group C (10.58 ± 0.69 ; 40.78 ± 2.50) and Group D (10.75 ± 0.73 ; 39.33 ± 3.69) at $P < 0.05$. However, there was no statistically significant difference in hemoglobin between Control compared to Group C and D at $P > 0.05$. In figure 5, there was statistically significant difference in MCV, MCH and MCHC between Group B (68.08 ± 4.03 ; 13.35 ± 0.82 ; 19.75 ± 0.95) compared to Control (54.50 ± 1.05 ; 14.68 ± 0.43 ; 28.65 ± 0.52), Group C (55.93 ± 1.19 ; 15.22 ± 0.31 ; 26.28 ± 0.56) and Group D (54.43 ± 1.10 ; 14.10 ± 0.63 ; 25.73 ± 0.67) at $P < 0.05$. However, there was no statistically significant difference in hemoglobin between Control compared to Group C and D respectively at $P > 0.05$.

Effect of Methanolic Extract of *Hibiscus sabdariffa* on some Hematological Parameters in Levodopa-Induced Anemia

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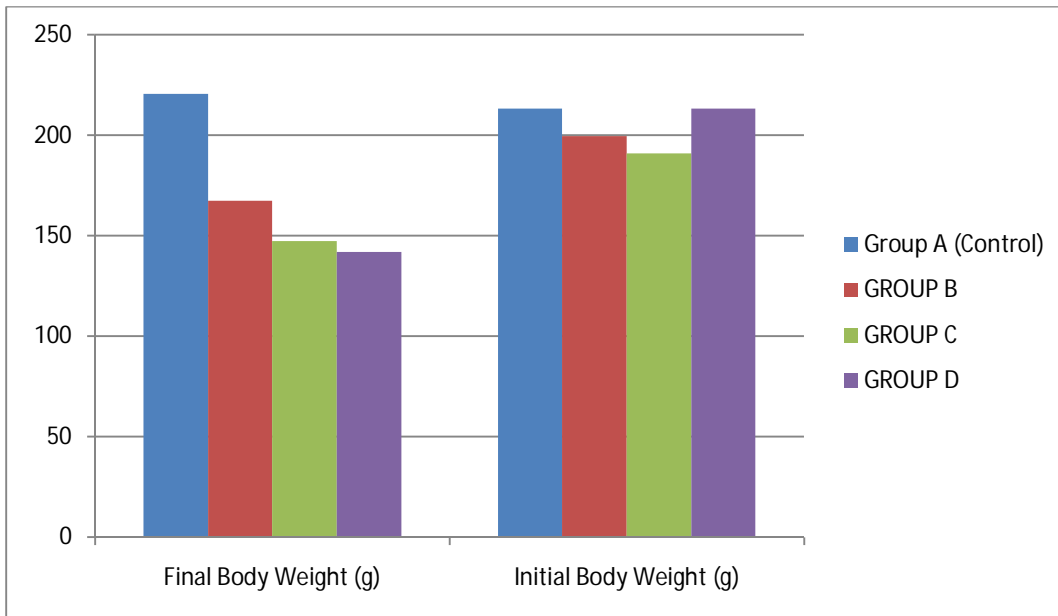


Figure 1: Effect of Methanolic Extract of *Hibiscus sabdariffa* on Body Weight Before and After Administration of Levodopa

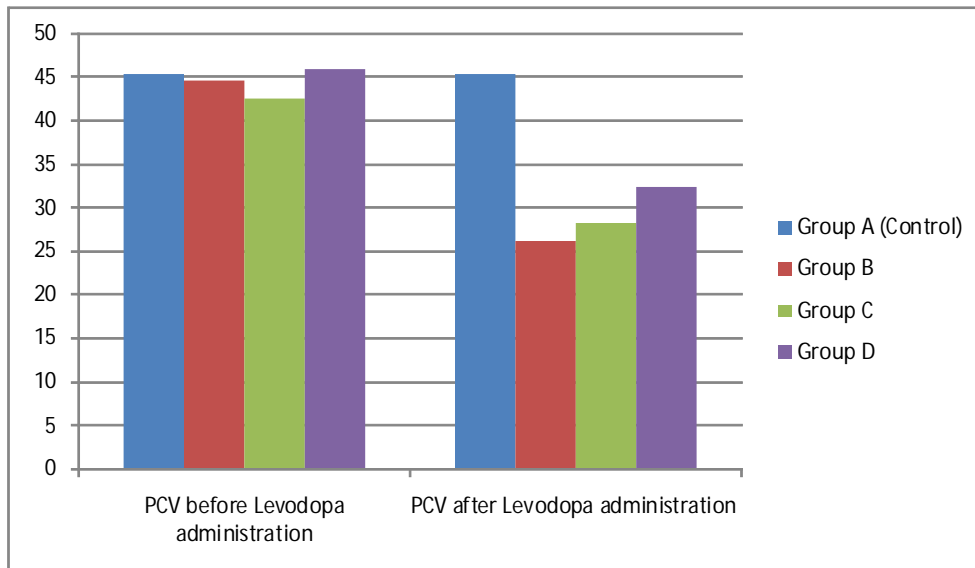


Figure 2: Packed Cell Volume (PCV) Before and After Administration of Levodopa

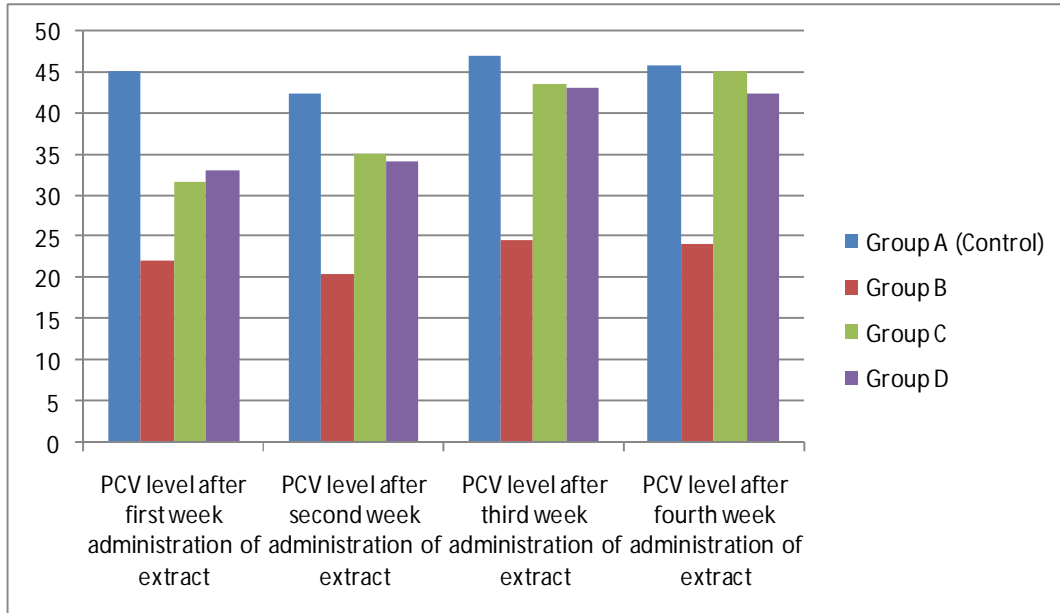


Figure 3: Weekly Effect of Methanolic Extract of *Hibiscus sabdariffa* on Packed Cell Volume (PCV) After Administration of Levodopa

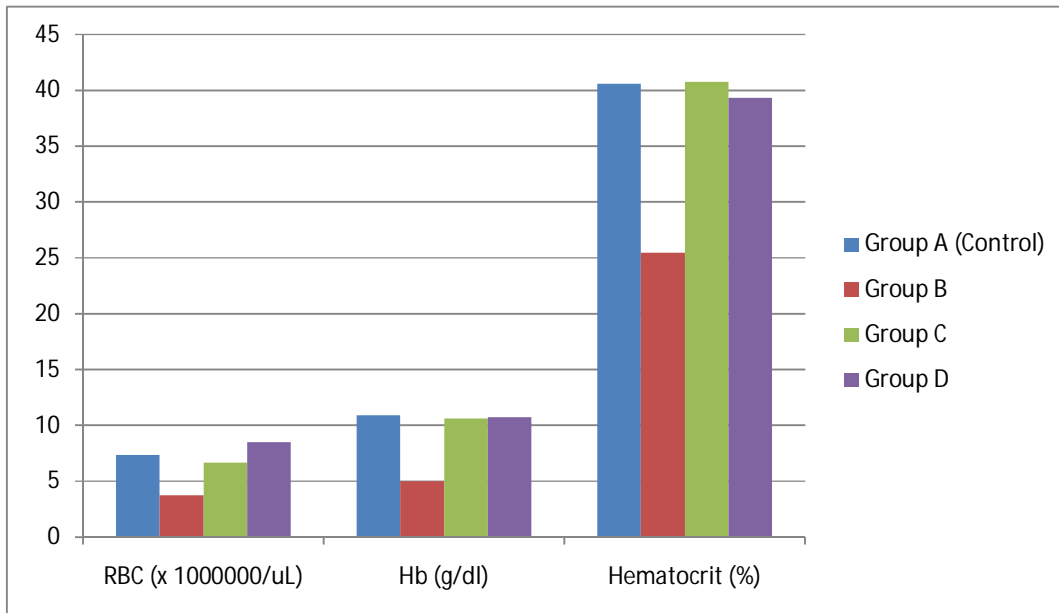


Figure 4: Effect of Methanolic Extract of *Hibiscus sabdariffa* on RBC, Hemoglobin and Hematocrit After Administration of Levodopa

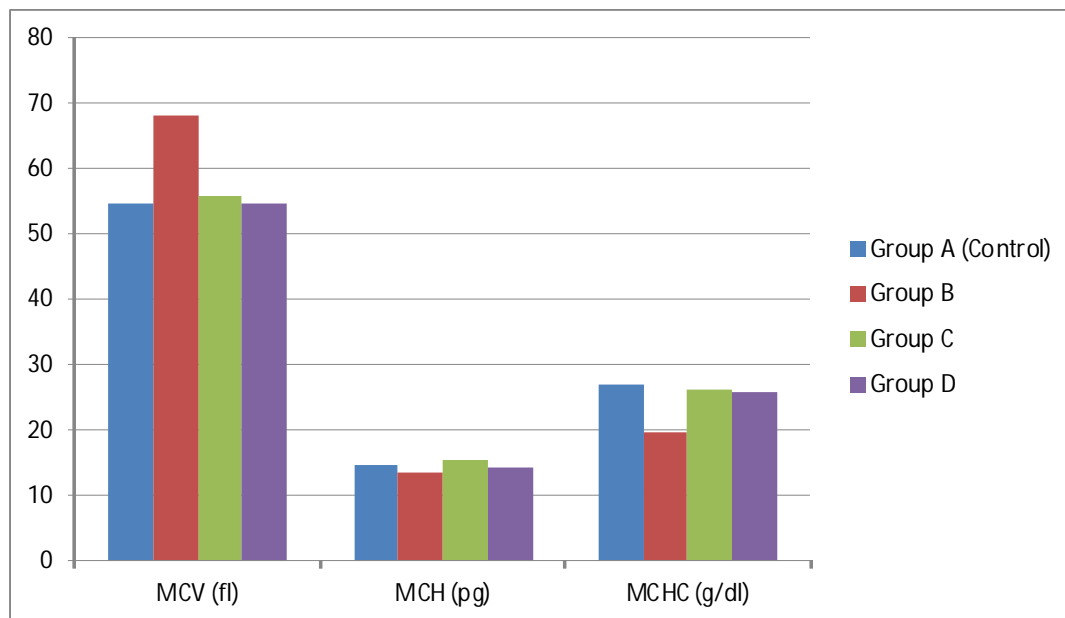


Figure 5: Effect of Methanolic Extract of *Hibiscus sabdariffa* on MCV, MCH and MCHC After Administration of Levodopa

DISCUSSION

In this present study, the body weight significantly decreased in animals treated with methanolic extract of *Hibiscus sabdariffa* suggesting that the extract caused body weight loss. Conversely, report has shown that rats that consumed aqueous extract of *Hibiscus sabdariffa* post-weaning significantly increased in body weight (Iyare and Nwagha, 2009). Roselle calyx has been shown to contain phytic acid, tannin and glycosides such as delphinidin-3-monoglucosides and delphinidin which are toxic to animal and human tissue at high concentrations (Morton, 1987; Ojokoh *et al.*, 2002), therefore, the reduction in body weight may be dependent on the dose concentration of the extract. Our findings in body weight corroborated with report by Emelike and Dapper (2013) which showed that oral administration of *Hibiscus sabdariffa* extract decreased body weight.

The PCV levels were significantly decreased after administration of levodopa in the experimental groups. Figure 3 showed that extract increased the PCV, RBC, hemoglobin concentration, hematocrit, MCV, MCH and MCHC levels back to normal after 4 weeks in this study. Several studies have shown that extract increased PCV, RBC, and hemoglobin concentration (Ologundudu *et al.*, 2007; Sini *et al.*, 2011; Olusola *et al.*, 2012; Emelike and Dapper, 2013). This increase in

hematological parameters could possibly be due to the antioxidative properties of anthocyanins which reduced loss of blood cells to lipid peroxidation (Andreas et al., 2000; Ologundudu *et al.*, 2007). Anthocyanins have also been implicated to induce renal secretion of erythropoietin, the most important signal for differentiation and multiplication of the pluripotent stem cells involved in blood cell formation (Heda and Bhatia, 1986; Kaur and Kapoor, 2005). Hemoglobin level increased significantly in the study, this may be due to iron. Iron has been reported to be present in significant amount in the whole extract of *Hibiscus sabdariffa* (Heda and Bhatia, 1986). Thus, suggesting that the extract increased these parameters after induction of anemia with levodopa. Hemoglobin concentration increased in this present study as expected, therefore the increase in MCH and MCHC levels could also involve the same mechanism of the iron content of *Hibiscus sabdariffa* as reported by Heda and Bhatia (1986), as the same is expected of MCV due to the increase in RBC count. Data obtained in this study therefore, suggests that methanolic extract of *Hibiscus sabdariffa* increases some hematological parameters in a dose-dependent manner in levodopa-induced anemic rats.

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