

# Effect of Chronic Administration of Hydromethanol Leaf Extract of *Helianthus annuus* on Erythrocytic Profile in Normal Rats

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**Abstract:** Effect of chronic administration of hydromethanol leaf extract of *Helianthus annuus* on erythrocytic profile in normal rat was investigated. The extract was prepared using cold maceration method and concentrated at 40°C. It was incorporated in feed at 2.5, 5 and 10 mg per 100 g feed. Albino rats were randomly assigned to 4 (A – D) groups of 14 rats each. Group A received standard feed while groups B – D received feed incorporated with *H. annuus* at 2.5, 5 and 10 mg/100 g feed, respectively. They were fed 10% of their body weight daily for 90 consecutive days. Blood samples were collected via retroorbital venous plexus from four rats selected at random from each group without replacement on day 30, 60 and 90 for the determination of erythrocytic profile. There was no significant ( $p > 0.05$ ) change on the erythrocytic profile of the various groups on day 30 and 60 while the packed cell volume and hemoglobin concentration of *H. annuus* treated groups were significantly ( $p < 0.05$ ) lower than group A rats on day 90. This study suggests that the chronic use of *Helianthus annuus* for up to 90 days in rat may lead to anemia and should be avoided.

**Keyword:** *Helianthus annuus*, chronic toxicity, erythrocytic profile, anemia, terpenes.

## INTRODUCTION

*Helianthus annuus* (sunflower) is relatively a primitive member of the family Asteraceae. It has worldwide distribution and usually found along water ways due to its mode of dispersal. It is used as an ornamental plant and the seed are widely used as sources of vegetable oil while seed meal serve as feedstuff for farm animals [1]. The plant is extensively used in ethnomedical practices of several cultures. *Helianthus annuus* leaves are used in folkloric medicine as diuretic, antidiabetic, expectorant, gastrointestinal stimulant, antimicrobial, analgesic agents etc [2-4]. Poultice of *H. annuus* leaves is applied on wound, snakebite and swelling [5]. Preliminary phytochemical studies on *H. annuus* have reported the presence of saponins, terpenes, flavonoids, tannins, alkaloids and glycoside [4, 6]. The presence of several essential oil in the leaves of *H. annuus*; 80% monoterpenes and other sesquiterpenes have been reported [2, 7]. The antispermatic [3], analgesic and anti-inflammatory [5], antidiabetic and antioxidant [4], antifertility [8], antidiarrheal and antihistaminic [9], antilithiatic [10] and antimicrobial [6] activities of *H. annuus* have been reported.

*Helianthus annuus* leaves contain high proportion of saponins and terpenes [4, 6] and its consumption for a

long period of time especially in diabetes mellitus management call for concern about its possible erythrocytic toxicity. Saponins causes hemolysis of red blood cell *in vivo* [11] This study investigated the effects of hydromethanol extract of *H. annuus* on erythrocytic profile in normal rat.

## MATERIAL AND METHODS

### Plant Collection and Extract Preparation

The leaf of *Helianthus annuus* was collected from the wild in Nsukka, Enugu state, Nigeria and identified by plant taxonomist. The leaves were dried under shed at ambient temperature (25 – 27°C). Hydromethanol extract of *H. annuus* was prepared using cold maceration method as described by Onoja and Anaga [4] and was referred to as hydromethanol leaf extract of *H. annuus* (HLEHA).

### Animals

Fifty six (56) female albino Wistar rats were used for the study. They were housed in aluminum cages in a well-ventilated room at ambient temperature (25 – 27°C) and natural light/darkness cycle. The rats were acclimatized for 2 weeks and the experimental protocol was approved by the Michael Okpara University of Agriculture, animal ethical committee.

### Feed Formulation

The hydromethanol extract of *H. annuus* was incorporated in the feed at the rate of 2.5, 5 and 10 mg

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per 10 g feed. The rate of incorporation was based on the percentage yield of the extract and doses used in previous studies [4]. Briefly, 500, 1000 and 2000 mg of the extract were weighed and dissolved in 2 litre of water each and later mixed with 2 kg of feed respectively. The resulting moist feed mixture was manually pelleted with the aid of the barrel of 5 ml syringe. The pellets were dried under mild sunlight for 2 days in harmattan condition. The pelleted feeds were used within 2 weeks to avoid mold growth.

### Experimental Design

The rats were randomly assigned to 4 (A – D) groups of 14 rats each and treated as follow: group A rats received standard pelleted feed while groups B – D received feed incorporated with HLEHA at 2.5, 5 and 10 mg/10 g feed, respectively. They were fed 10% of their body weight daily for 90 consecutive days. They finished their feed each day and a deep feeding troughs were used to avoid feed wastage. On weekly bases, the rats were weighed and feeding was adjusted to accommodate the weight change. The hematological profiles of the rats were determined on day 30, 60 and 90 of treatment. Four animals were randomly selected from each group at the timed interval and blood samples were collected from the retroorbital venous plexus with the aid of heparinized capillary tube into ethylenediaminetetraacetic acid (EDTA) bottle. The parameters were determined within 24 h of blood sample collection.

The method of Dacie and Lewis [12] was employed in red blood cell (RBC) count; hematocrit method [13] was used in packed cell volume (PCV) estimation while hemoglobin (HB) concentration was estimated with cyanomethemoglobin method using Drabkin's reagent [13]. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) was calculated as described by [14].

### Data Analysis

The data were analyzed using one-way analysis of variance (ANOVA), variant means were separated least significant difference (LSD) and significance was accepted at  $p < 0.05$  with statistical package for social sciences (SPSS) version 21.0

### RESULTS

Generally there was no significant ( $p > 0.05$ ) change in the value of all the parameters determined on day 30 and 60 except for MCHC on day 30. There was no significant ( $p > 0.05$ ) difference in the RBC values of the treatment groups (Table 1). At day 90, the PCV of Groups C and D were significantly ( $p < 0.05$ ) lower than the value of group A (Table 2). The Hb concentration of HLEHA -treated groups were significantly ( $p < 0.05$ ) reduced compared to the rats that received standard feed on day 90 (Table 3). The MCV of group B was significantly ( $p < 0.05$ ) lower while

**Table 1: Effect of HLEHA on RBC in Normal Rats**

Group	Treatment	Mean total RBC count ( $\times 10^6/\mu\text{L}$ ) $\pm$ SEM		
		Day 30	Day 60	Day 90
A	Standard feed	9.49 $\pm$ 0.67	8.75 $\pm$ 0.22	8.79 $\pm$ 0.36
B	HLEHA 2.5 mg/10 g feed	9.68 $\pm$ 0.27	9.45 $\pm$ 0.36	9.74 $\pm$ 0.40
C	HLEHA 5 mg/10 g feed	8.95 $\pm$ 0.36	8.43 $\pm$ 0.21	9.00 $\pm$ 0.47
D	HLEHA 10 mg/10 g feed	8.74 $\pm$ 0.37	8.75 $\pm$ 0.21	8.51 $\pm$ 0.47

$P > 0.05$  when compared to standard feed group HLEHA = hydromethanol leaf extract of *H. annuus*; SEM = standard error of mean; RBC = Red blood cell.

**Table 2: Effect of HLEHA on PCV in Normal Rats**

Group	Treatment	Mean PCV (%) $\pm$ SEM		
		Day 30	Day 60	Day 90
A	Standard feed	47.00 $\pm$ 1.90	44.38 $\pm$ 1.95	45.38 $\pm$ 0.85
B	HLEHA 2.5 mg/10 g feed	47.63 $\pm$ 0.43	45.00 $\pm$ 1.47	43.50 $\pm$ 1.04
C	HLEHA 5 mg/10 g feed	44.88 $\pm$ 0.43	44.00 $\pm$ 1.08	42.00 $\pm$ 1.29*
D	HLEHA 10 mg/10 g feed	44.25 $\pm$ 1.69	44.50 $\pm$ 1.44	41.63 $\pm$ 0.99*

\* $p < 0.05$  compared to standard feed group; HLEHA = hydromethanol leaf extract of *H. annuus*; PCV = packed cell volume; SEM = standard error of mean.

group C and D were not significant ( $p > 0.05$ ) compared to group A on day 90 (Table 4). Also, on day 90, MCH of groups B and C were decreases significantly ( $p < 0.05$ ) compared to group A (Table 5). The MCHC of group D was significantly higher ( $p < 0.05$ ) compared to group A on day 30 while the was no significant ( $p > 0.05$ ) difference in the various treatment groups on days 60 and 90 (Table 6).

## DISCUSSION

The choice of incorporating HLEHA in the feed was to avoid stress of daily oral administration of the extract throughout the duration of the study. The study showed hematological changes associated with chronic administration of HLEHA to rats. Feeding of the rats with extract-treated feed did not produce a significant ( $p > 0.05$ ) hematological change on the first 60 days of

**Table 3: Effect of HLEHA on HB in Normal Rats**

Group	Treatment	Mean HB (g/dl) $\pm$ SEM		
		Day 30	Day 60	Day 90
A	Standard feed	14.49 $\pm$ 0.55	14.87 $\pm$ 0.36	16.08 $\pm$ 0.34
B	HLEHA 2.5 mg/10 g feed	14.29 $\pm$ 0.46	15.35 $\pm$ 0.27	14.72 $\pm$ 0.28*
C	HLEHA 5 mg/10 g feed	14.34 $\pm$ 0.16	14.17 $\pm$ 0.30	14.60 $\pm$ 0.65*
D	HLEHA 10 mg/10 g feed	14.70 $\pm$ 0.57	15.25 $\pm$ 0.22	14.84 $\pm$ 0.39*

\* $p < 0.05$  compared to standard feed group; HLEHA = hydromethanol leaf extract of *H. annuus*; HB = hemoglobin; SEM = standard error of mean.

**Table 4: Effect of HLEHA on MCV in Normal Rats**

Group	Treatment	Mean MCV (fl) $\pm$ SEM		
		Day 30	Day 60	Day 90
A	Standard feed	50.00 $\pm$ 2.58	50.89 $\pm$ 2.99	51.87 $\pm$ 2.17
B	HLEHA 2.5 mg/10 g feed	49.33 $\pm$ 1.35	47.66 $\pm$ 0.65	44.90 $\pm$ 2.07*
C	HLEHA 5 mg/10 g feed	50.36 $\pm$ 1.82	52.35 $\pm$ 2.07	46.84 $\pm$ 1.23
D	HLEHA 10 mg/10 g feed	50.77 $\pm$ 1.69	51.35 $\pm$ 3.82	48.96 $\pm$ 1.72

\* $p < 0.05$  compared to standard feed group; HLEHA = hydromethanol leaf extract of *H. annuus*; MCV = Mean corpuscular volume; SEM = standard error of mean.

**Table 5: Effect of HLEHA on MCH in Normal Rats**

Group	Treatment	Mean MCH (pg) $\pm$ SEM		
		Day 30	Day 60	Day 90
A	Standard feed	15.43 $\pm$ 0.83	17.05 $\pm$ 0.78	18.37 $\pm$ 0.63
B	HLEHA 2.5 mg/10 g feed	14.83 $\pm$ 0.82	16.33 $\pm$ 0.78	15.19 $\pm$ 0.62*
C	HLEHA 5 mg/10 g feed	16.09 $\pm$ 0.61	16.87 $\pm$ 0.71	16.30 $\pm$ 0.72*
D	HLEHA 10 mg/10 g feed	16.93 $\pm$ 0.99	17.55 $\pm$ 0.92	17.43 $\pm$ 0.48

\* $p < 0.05$  compared to standard feed group; HLEHA = hydromethanol leaf extract of *H. annuus*; MCH = Mean corpuscular hemoglobin; SEM = standard error of mean.

**Table 6: Effect of HLEHA on MCHC in Normal Rats**

Group	Treatment	Mean MCHC (%) $\pm$ SEM		
		Day 30	Day 60	Day 90
A	Standard feed	30.85 $\pm$ 0.11	33.61 $\pm$ 0.82	35.45 $\pm$ 0.46
B	HLEHA 2.5 mg/10 g feed	30.00 $\pm$ 0.95	34.27 $\pm$ 1.60	35.35 $\pm$ 1.56
C	HLEHA 5 mg/10 g feed	31.95 $\pm$ 0.24	32.22 $\pm$ 0.39	34.77 $\pm$ 1.02
D	HLEHA 10 mg/10 g feed	33.28 $\pm$ 1.12*	34.33 $\pm$ 0.75	35.71 $\pm$ 1.37

\* $p < 0.05$  compared to standard feed group; HLEHA = hydromethanol leaf extract of *H. annuus*; SEM = standard error of mean; MCHC = Mean corpuscular hemoglobin concentration.

treatment. On day 90, mild normocytic hypochromic anemia was recorded in the extract treated groups which could be attributed to the phytochemical constituents of *H. annuus*. Ndam *et al.* [15] reported that the physiological, biochemical and pharmacological activities of medicinal plants is related to their phytoconstituents. *Helianthus annuus* has been reported to contain terpenes, alkaloids, saponins and other phytoconstituents [16]. Some of the terpenes and saponins isolated from *H. annuus* leaves and oil include; D-limonene,  $\alpha$ -pinene, menthol, sabinene, germacrene D, camphene,  $\beta$ -pinene, isobornyl acetate,  $\alpha$ -terpineol etc [2]. Mendanha *et al.* [17], reported the *in vitro* hemolytic activity of terpenes; nerolidol, 1,8-cineole,  $\alpha$ -terpineol, pulegone,  $\alpha$ -limonene etc, against normal human erythrocyte. The hemolytic activities of saponins have been proposed by researchers [18]. The reported hemolytic activities of saponins and terpenes shorten the life span of erythrocyte and predispose the affected animal to hemolytic anemia [19]. The mild anemia recorded in this study may be due to the low dose of *H. annuus* that was used in the study. High dose may cause a more severe anemia even within shorter period [20]. The absence of significant difference in the red blood cell count may be due to regenerative response of the erythropoietic tissue [14].

This study suggests that the chronic use of *H. annuus* leaf for up to 90 days in rat may lead to hemolytic anemia.

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## DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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