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Research Article

EFFECT OF ISOLATED ERGOSTEROL CONSTITUENT OF *Portulaca oleracea* ON HAEMATOLOGICAL PARAMETERS IN MALE ALBINO RATS

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ABSTRACT

The effect of oral administration of isolated ergosterol constituent of *Portulaca oleracea* at doses of 0.50 mg/kg BW and 0.75 mg/kg BW on the haematological parameters of albino rats were investigated. The isolated compound was administered on daily basis for 25 days and blood samples were collected for analyses.

Treatment of rats with 0.50 mg/kg BW and 0.75 mg/kg BW of ergosterol caused significant ($p<0.05$) increase in the platelet, red blood cell (RBC) and total white blood cell (TWBC) counts as well as significant increase ($p<0.05$) in the packed cell volume (PCV) value relative to their respective controls. Treatment of rats with 0.75 mg/kg BW of erogesterol caused significant ($p<0.05$) increase and decrease respectively in haemoglobin concentration (Hb) and eosinophil values relative to their respectively controls.

These findings on the haematological parameters suggest that the changes in blood chemistry of the treated rats were due to the isolated ergosterol constituent of *Portulaca oleracea.*

Keywords: Ergosterol, Red blood cells, Total white blood cells, Packed cell volume, Albino rats.

INTRODUCTION

Portulaca oleracea belongs to the family of *Portulacaceae*. It is commonly called Purslane in English language, "Babbajibji" in Hausa language and "Esan omode" or "Papasan" in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long (Burkill, 1997).

It is used medicinally in Ghana for heart-palpitations (Johnson, 1997). The plant is used as a diuretic in Nigeria (Ainslie, 1973). A tisane of the plant is drunk in Trinidad as a vermifuge (Wong, 1976).

At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the foetus (Vermmer, 1976).

It has been reported that aqueous and methanolic extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in in-vitro preparations (Oyedeji *et al,* 2007).

The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue (Wang et al, 2007), anti-inflammatory effects (Xiang et al, 2005) and wound-healing activity (Rashed *et al,* 2003). Parry et al (1987) also reported the skeletal muscle relaxant effect of the plant.

This study aims at investigating the effect of isolated ergosterol constituent of Portulaca oleracea on haematological parameters in male albino rats.

MATERIALS AND METHODS

Experimental Animals

Adult male albino rats weighing between 150 g and 250 g bred in the Pre-clinical Animal House of the College of Medicine, University of Ibadan were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle and had free access to feed and water; and were acclimatized for two weeks to laboratory conditions before the commencement of the experiments. All experiments were carried out in compliance with the recommendations of Helsinki's declaration on guiding principles on care and use of animals.

Plant Material

Fresh specimens of *Portulaca oleraceae* were collected from the Botanical Garden of the Forestry Research Institute of Nigeria, Jericho, Ibadan, and was authenticated in the above named institute where a voucher specimen (No FHI 108334) was deposited.

Extraction, Fractionation and Isolation of Constituents of *Portulaca oleracea*

About 3.2 kg of air-dried specimen of *Portulaca oleracea* was cold-extracted in methanol for 72 hours. The mixture was filtered using a wire-gauze and a sieve with tiny pores (0.25 mm) and concentrated at room temperature by exposing the extract for six days. The resulting solution was then placed in the oven at a reduced temperature (50^0C).

The methanolic extract was then preabsorbed with silical gel and placed in the oven at a reduced temperature (50^0C) overnight and then subjected to open column chromatography on silical gel (F_{254}, 50-200 mesh, E. Merck) for fractionation. The solvents (mobile phases) were hexane (non-polar), ethylacetate (partially polar) and methanol (polar). The gradients of the mobile phases involved hexane with an increasing percentage of ethylacetate (hexane/ethylacetate mixture) and then ethylacetate with an increasing percentage of methanol (ethylacetate/methanol mixture) as shown below:

Hexane		Ethylacetate	Methanol
100% (50 mL)	:	0% (0 mL)	
90% (45 mL)	:	10% (5 mL)	
80% (40 mL)	:	20% (10 mL)	
70% (35 mL)	:	30% (15 mL)	
60% (30 mL)	:	40% (20 mL)	
50% (25 mL)	:	50% (25 mL)	
40% (20 mL)	:	60% (30 mL)	
30% (15 mL)	:	70%(35 mL)	
20% (10 mL)	:	80% (40 mL)	
10% (5 mL)	:	90% (45 mL)	
0% (0 mL)	:	100% (50 mL)	: 0% (0 mL)
		90% (45 mL):	: 10% (5 mL)
		80% (40 mL)	: 20% (10 mL)
		70% (35 mL)	: 30% (15 mL)

60% (30 mL)	: 40% (20 mL)
50% (25 mL)	: 50% (25 mL)
40% (20 mL)	: 60% (30 mL)
30% (15 mL)	: 70% (35 mL)
20% (10 mL)	: 80% (40 mL)
10% (5 mL)	: 90% (45 mL)
0% (0 mL)	: 100% (50 mL)

Thin Layer Chromatography (TLC)

The 21 fractions were spotted on precoated plates of silica gel GF_{254} (20 x 20, 0.5 mm thick; E. Merck) using capillary tubes. The spotted TLC plates were developed in a tank that contained a mixture of ethylacetate/methanol (9:1) as the mobile phases.

The TLC plates were then examined under the ultraviolet (UV) light at a wavelength of 365 nm and the well-defined spots of the components were then revealed by the UV light. Fractions with similar relative fronts or retention or retardation factors (R_f value) were then pooled or bulked together, this then reduced the number of fractions to five (fractions 1, 2, 3, 4, 5) .

$$R_f = \frac{\text{distance compound has moved from origin}}{\text{Distance of solvent front from}}$$

The TLC analysis of all the fractions indicated fraction 2 as the fraction that contains many components.

This fraction 2 was further subjected to open column chromatography and eluted using hexane and chloroform (Hexane: Chloroform 50:50) as mobile phases to produce another 46 fractions (Isolated compounds). Isolated compound 7 upon standing overnight gave regular – shaped crystals which were separately washed with hexane and sent for UV, IR and NMR analyses.

Spectroscopy

The quantitative estimation of the isolated compound was obtained by the ultraviolet (UV) spectrophotomety. The infrared and the nuclear magnetic resonance (NMR) analyses were to identify the nature and to obtain the formulae of the isolated compounds.

Ultraviolet (UV) analysis

The UV spectra of the isolated compound was recorded in Chloroform in Genysis 32010 (thermoelectron coupling) spectrophotometer at the Central Research Laboratory, Ladoke Akintola University of Technology, Ogbomoso.

Infrared (IR) analysis

The IR spectra of the isolated compounds were recorded in Nujol on Spectrum II BX FTIR (Perkin Elmer) spectrophotometer at the Central Research Laboratory, University of Ibadan.

Nuclear Magnetic Resonance (NMR) analysis

The ^{1}H-NMR spectra was recorded at 200MH2 in CDCl3 on a Varian-Mercury nuclear magnetic resonance spectrophotometry using tetramethysilane (TSM) as an internal standard at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife.

The ^{1}H-NMR shifts was calculated for the isolated compound using the Advanced Chemistry Development (ACD) software for further confirmation of the structure of the isolated compound.

Acute Toxicity Test of the Isolated Compound

The acute toxicity test of the isolated compound of *Portulaca oleracea* was evaluated in albino mice as described by Miller and Tainter (1994). Fifteen adult male mice weighing between 20-22g were divided into five mice per group for the isolate. Three doses of the isolate: 0.5 mg/kg BW, 2.5 mg/kg BW and 5 mg/kg BW were orally given to the animals. The control group mice (n=5) received 0.2 ml of distilled water. The animals were observed for seven days for behavioural changes and mortality.

Experimental Design

Fifteen animals were randomly divided into three groups with each group consisting of five rats. The three groups were subjected to the following oral daily treatments for 25 days:

Group I rats received 0.50 mg/kg BW of ergosterol.

Group II rats received 0.75 mg/kg BW of ergosterol.

Group III rats received 0.5 ml of distilled water as the control group.

Collection of Blood Samples

Blood samples were collected through the medial cantus into EDTA bottles for haematological analyses.

Determination of Haematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the improved Neubauer haemocytometer method. The haemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the micro-haematocrit method according to Dacie and Lewis (1991). Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells (Mitruka and Rawnsley, 1977). Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to Jain (1986).

Statistical Analysis

The mean and standard error of mean (S.E.M) were calculated for all values. Comparison between the control and experimental groups was done using one-way analysis of variance (ANOVA) with least significant difference (LSD). Differences were considered statistically significant at $p<0.05$.

RESULTS

Acute Toxicity

No mortality and changes in behaviour were observed in all the treated and control groups. Hence lower doses of the isolated compound were used for this study.

Spectral Analyses

The characterized name of compound 7 that was sent for spectral analyses is ergosterol. The chemical identity and the structural elucidation of this compound was obtained based on the spectroscopical analyses.

The UV spectrum of compound 7 (Figure 1) shows absorbance at 205 nm, 238 nm, 268 nm, 352 nm which is indicative of the presence of chromophore.
The IR spectrum of compound 7 (Figure 2) shows signals at 2832.74 cm^{-1} corresponding to C-H stretching vibrations, 1747.93 cm^{-1} for C= O. stretching vibrations, 1453.94 cm^{-1} for C-H deformations, 1469.97 cm^{-1} for C-H deformations, and 1376.97 cm^{-1} for C-H deformations.
Further justification to the structure of compound was obtained from the ^{1}H-NMR spectrum of compound 7 (Figure 3). Details of the ^{1}H-NMR of compound 7 is presented in Table 1.

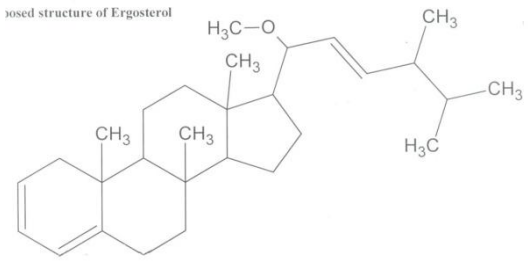
All these facts point to the proposed structure as ergosterol.

Table 1: ^{1}H-NMR chemical shift (δ) data of compound 7 (Ergosterol)

S/No	δH (ppm)	Multiplicity	J (MH_Z)
1	2.02	Singlet	-
2	5.64	Singlet	-
3	5.66	Singlet	-
4	5.38	Multiplet	-
5	-	-	-
6	1.84	Singlet	-
7	1.50	Singlet	-
8	-	-	-
9	2.36	Triplet	20
10	-	-	-

11	1.10	Singlet	-
12	1.50	Singlet	-
13	-	-	-
14	2.02	Triplet	20
15	1.10	Singlet	-
16	2.10	Singlet	-
17	1.82	Singlet	-
18	3.48	Doublet	10
19	4.20	Double doublet	20
20	4.36	Double doublet	20
21	2.10	Singlet	-
22	1.62	Singlet	-
23	0.98	Singlet	-
24	0.90	Singlet	-
25	0.90	Singlet	-
26	-	-	-
27	0.90	Singlet	-
28	0.91	Singlet	-
29	0.98	Singlet	-

The proposed structure of ergosterol is shown below:



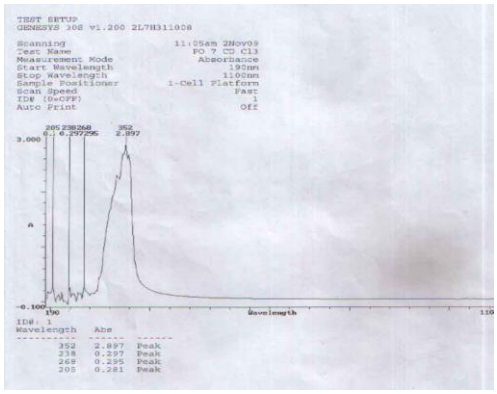


Figure 1: UV spectrum of compound 7 (ergosterol)

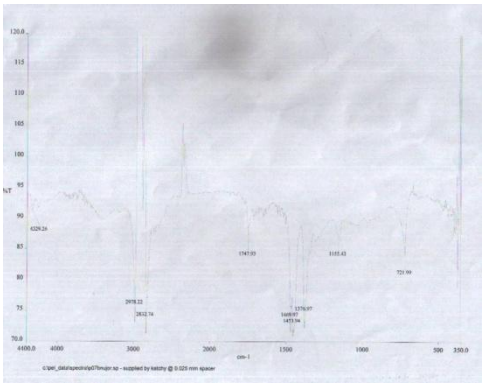


Figure 2: IR spectrum of compound 7 (ergosterol).

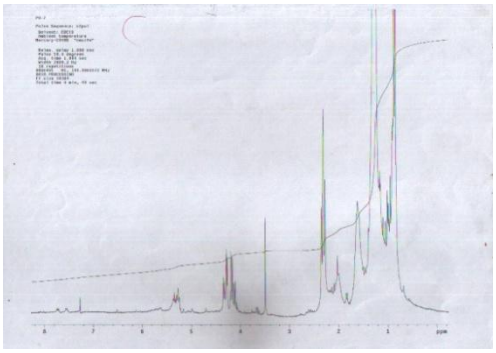


Figure 3: ^{1}H-NMR spectrum of compound 7 (ergosterol)

Effect of ergosterol on haematological parameters

The effect of ergosterol at various doses on the haematological parameters of albino rats after treatment of rats for 25 days is shown in Table 2.

Treatment of rats with 0.50 mg/kg BW and 0.75 mg/kg BW of ergosterol caused no significant ($p>0.05$) change in the MCV, MCHC, MCH, neutrophiol, lymphocyte and monocyte values relative to their respective contrhmols. Treatment of rats with 0.50 mg/kg BW and 0.75 mg/kg BW of ergosterol caused significant ($p<0.05$) increase in the platelet, RBC and TWBC counts relative to their respective controls. Treatment of rats with 0.5 mg/kg BW and 0.75 mg/kg BW of ergosterol resulted in significant ($p<0.05$) increase in the PCV value relative to the control. Treatment of rats with 0.75 mg/kg BW of ergosterol caused significant ($p<0.05$) increase and decrease respectively in the Hb and eosinophil values relative to their respective controls.

Table 2:Effect of Ergosterol on Haematological Parameters after reatment of Rats for 25 Days (n = 5,*p<0.05)

Parameters	Control	0.50 mg/kg	0.75 mg/kg
PCV (%)	41.80 ± 1.03	46.5 ± 0.29*	49.08 ± 1.03*
Hb (g/dl)	13.90 ± 0.41	15.2 ±0.21	16.30 ± 0.60*
RBC (x10^6/ µl)	7.07 ± 0.27	7.72 ± 0.02*	8.14 ± 0.23*
MCV (FL)	59.10 ± 0.78	60.10 ± 0.44	61.10 ± 0.50
MCHC (g/dl)	33.40 ± 0.25	32.70 ± 0.29	32.70 ± 0.58
MCH (pg)	19.90 ± 0.32	19.70 ± 0.32	20.00 ± 0.20
TWBC (x10^3/ µL)	11.00 ± 0.42	15.00 ± 0.11*	17.00 ± 1.01*
Platelets (10^5/ µL)	1.10 ± 0.10	1.40 ± 0.12*	1.50 ± 0.12*
Neutrophils (%)	27.00 ± 0.41	28.00 ± 0.41	24.00 ± 7.08
Lymphocytes (%)	68.00 ± 0.41	67.50 ± 0.29	66.30 ± 0.85
Eosinophils (%)	2.25 ± 0.48	1.75 ± 0.25	0.75 ± 0.25*
Monocytes (%)	2.75 ± 0.63	2.00 ± 0.00	2.00 ± 0.41

DISCUSSION

It was observed that the highest dose of the isolated compound caused no mortality or behavioural changes in all the treated animals which indicates that the isolate has wide safety margins.

The effect of ergosterol at doses of 0.50 mg/kg BW and 0.75 mg/kg BW on the haematological parameters of albino rats after treatment for 25 days is shown in Table 2.

The study has revealed that ergosterol caused significant increase in the RBC and Hb values. This suggests that ergosterol has the potential to stimulate erythropoietin release from the kidneys with a resultant increase in the rate of RBC production (erythropoiesis) which could ultimately induce polycythemia, since it has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anaemic conditions while higher values are suggestive of polycythemia (American Diabetes Association, 2000). It could also indicate that there was an enhancement in the oxygen-carrying capacity of blood and the

amount of oxygen delivered to the tissues since RBC and haemoglobin are very important in transferring respiratory gases (De Gruchy, 1976). Contrary report was given by Adedapo *et al* (2007) in *P.amarus* treated rats.

The significant increase in the TWBC count induced by ergosterol may imply an enhancement in the ability of the body to respond to infections. Similar report was given by Adewusi and Afolayan (2009) in *Pelargonium reniforme* extract treated rats.

Ergosterol caused significant increase in platelet count which could be an indication that it has the potential to stimulate thrombopoietin production (Li *et al.,* 1999) with a resultant enhancement in the haemostatic capability of the blood since platelets mediate in the blood – clotting mechanism.

Ergosterol caused no significant changes in the MCV and MCH values which could be an indication of absence of macrocytic anaemia since increased MCV an MCH values are known to be indicative of macrocytic anaemia. Also, ergosterol caused non- significant change in the MCHC value which suggest an absence of hereditary spherocytosis since MCHC values are known to be elevated in hereditary spherocytosis.

The insignificant change in neutrophil count caused by ergosterol probably indicates that the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (Phagocytosis) has not been compromised. The non-significant change in lymphocyte count suggests that the acquired immune response of the body has not been compromised by ergosterol; while the insignificant change in monocyte count probably indicates that the phagocytic function of the body has not been compromised by ergosterol. The significant decrease in eosinophil count probably indicates that the anti-allergic and anti-parasitic infectious responses of the body have been compromised by ergosterol.

In conclusion, this study has shown that isolated ergosterol constituent of *Portulaca oleracea* has some beneficial and harmful potentials on the blood chemistry of albino rats. However, the effect of ergosterol on human blood chemistry is unknown, nevertheless, considering these findings in animal model, it is recommended that caution should be exercised in the consumption of ergosterol.

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