

EFFECT OF ISOLATED TETRACYCLIC STEROID CONSTITUENT OF *PORTULACA OLERACEA* ON HAEMATOLOGICAL PARAMETERS IN ALBINO RATS

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Received: 01 Apr 2013, Revised and Accepted: 06 May 2013

ABSTRACT

Objective: This study was designed to evaluate the effect of isolated tetracyclic steroid constituent of *Portulaca oleracea* on haematological parameters in albino rats.

Methods: The isolated compound was administered on daily basis for 25 days at doses of 0.50 mg/kg and 0.75 mg/kg; blood samples were collected for analysis. Distilled water (0.5 ml) served as the control. Red Blood Cell (RBC) and Total White Blood Cell (TWBC) counts were determined using haemocytometer. Differential leucocyte count was done using the Schilling method. Data were analysed using ANOVA at $p < 0.05$.

Results: Treatment of rats with 0.50 mg/kg BW and 0.75 mg/kg BW of tetracyclic steroid caused significant ($p < 0.05$) increase in the packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell (RBC) values relative to their respective controls. Treatment of rats with 0.75 mg/kg BW of tetracyclic steroid caused significant ($p < 0.05$) increase in the mean corpuscular volume (MCV) relative to the control.

Conclusion: These findings on haematological parameters suggest that the changes in blood chemistry of the treated rats was due to the isolated tetracyclic steroid constituent of *Portulaca oleracea*.

Keywords: Tetracyclic steroid, 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, "Babbajibi" in Hausa language and "Esan omode or Papan" in Yoruba language. It is a fleshy annual herb, much-branched and attaining 30 cm long [1].

It is used medicinally in Ghana for heart-palpitations [2]. The plant is used as a diuretic in Nigeria [3]. A tisane of the plant is drunk in Trinidad as a vermifuge [4].

At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the foetus [5].

It has been reported that aqueous and methanolic extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations [6].

The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue [7], anti-inflammatory

effects [8] and wound-healing activity [9]. [10] also reported the skeletal muscle relaxant effect of the plant.

This study aims at investigating the effect of isolated tetracyclic steroid constituent of *Portulaca oleracea* on the 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.

Table

| 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) |

Twenty-one fractions were obtained after the column chromatographic procedure.

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 °C).

The methanolic extract was then preabsorbed with silical gel and placed in the oven at a reduced temperature (50 °C) overnight and then subjected to open column chromatography on silical gel (F₂₅₄, 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 table:

Thin Layer Chromatography (TLC)

The 21 fractions were spotted on precoated plates of silica gel GF₂₅₄ (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)

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

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 29 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) spectrophotometry. The infrared and the nuclear magnetic resonance (NMR) analyses were to identify the nature and to obtain the formulae of the isolated compounds.

(i) 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, Ladoko Akintola University of Technology, Ogbomosho.

(ii) Infrared (IR) analysis

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

(iii) Nuclear Magnetic Resonance (NMR) analysis

The ¹H-NMR spectra was recorded at 200MHZ and the ¹³C-NMR spectra at 50MHz in CDCl₃ on a Varian-Mercury nuclear magnetic resonance spectrophotometry using tetramethylsilane (TSM) as an

internal standard at the Central Science Laboratory, Obafemi Awolowo University, Ile-Ife.

The ¹H-NMR and ¹³C-NMR shifts were 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 [11]. 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 tetracyclic steroid.

Group II rats received 0.75 mg/kg BW of tetracyclic steroid.

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 [12], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the micro-haematocrit method according to [13]. Schilling method of differential leucocyte count was used to determine the distribution of the various white blood cells [14]. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to [12].

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

(i) 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.

(ii) Spectral Analyses

The characterized name of compound 29 that was sent for spectral analyses is tetracyclic steroid. The chemical identity and the structural elucidation of this compound was obtained based on the spectroscopical analyses.

The UV spectrum of compound 29 (Figure 1) shows absorbance at 316 nm, 256 nm, 238 nm and 196 nm, this corresponds to $\pi \rightarrow \pi^*$ transition and $n \rightarrow \pi^*$ transition.

The IR spectrum of compound 29 (Figure 2) shows signals at 2911.93 cm⁻¹ corresponding to C-H stretching vibrations, 1712.74 cm⁻¹ for C=O stretching vibrations, 1650.27 cm⁻¹ for C=C stretching vibrations, 1462.15 cm⁻¹ for C-H deformations and 1022.04 cm⁻¹ for C=O stretching vibrations.

Further justification to the structure of compound 29 was obtained from the NMR spectra of compound 29 (Figures 3 and 4).

The $^1\text{H-NMR}$ of compound 29 indicated the presence of 7 methyl groups, characteristics fingerprint of tetracyclic triterpenes, there is also one prominent olefinic proton.

The $^{13}\text{C-NMR}$ also corroborated the proposed structure. Signals at 21.31 corresponds to a methyl group attached to a carbonyl functional group, other methyl signals are seen at 22.9 (q), 22.9 (q),

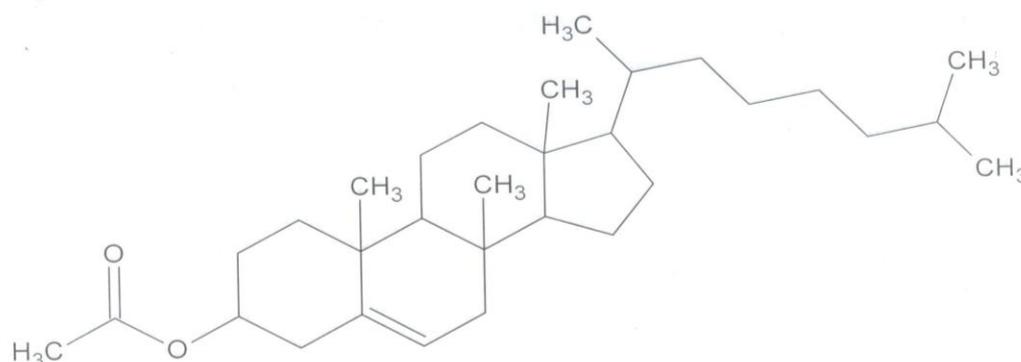
19.0 (q), 14.4(q), 12.2(q), 19.0 (q) indicating 6 methyls groups. The presence of olefinic carbons are indicated by the $^{13}\text{C-NMR}$ corresponding to the signals at 122.0 (s) and 136.0 (d). The carbonyl carbon also shows the characteristics $^{13}\text{C-NMR}$ at 179.8 ppm. The clustering of signals within 20 and 50 is a characteristics fingerprint of a steroid. The signals at 72.1 ppm corresponds to a CHO assigned to C-3. Details of the $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ of compound 29 is presented in Table 1.

All these facts point to the proposed structure as tetracyclic steroid.

Table 1: $^1\text{H-NMR}$ and $^{13}\text{C-NMR}$ chemical shift (δ) data of compound 29 (tetracyclic steroid)

| $^1\text{H-NMR}$ | | | $^{13}\text{C-NMR}$ | | |
|------------------|------------------------|--------------|---------------------|------------------------|--------------|
| S/No | δH (ppm) | Multiplicity | S/No | δC (ppm) | Multiplicity |
| 1 | 1.90 | Doublet | 1 | 36.386 | Triplet |
| 2 | 1.50 | Singlet | 2 | 26.242 | Triplet |
| 3 | - | - | 3 | 72.098 | Doublet |
| 4 | 2.00 | Singlet | 4 | 37.456 | Triplet |
| 5 | - | - | 5 | 122.012 | Singlet |
| 6 | 5.35 | Singlet | 6 | 136.012 | Doublet |
| 7 | 2.00 | Singlet | 7 | 39.990 | Triplet |
| 8 | - | - | 8 | 32.175 | Singlet |
| 9 | 2.08 | Singlet | 9 | 46.036 | Doublet |
| 10 | - | - | 10 | 36.386 | Singlet |
| 11 | 1.90 | Doublet | 11 | 19.255 | Triplet |
| 12 | 1.50 | Singlet | 12 | 39.990 | Triplet |
| 13 | - | - | 13 | 39.990 | Singlet |
| 14 | 2.00 | Singlet | 14 | 56.984 | Doublet |
| 15 | 1.00 | Doublet | 15 | 24.952 | Triplet |
| 16 | 1.90 | Doublet | 16 | 26.242 | Triplet |
| 17 | 1.70 | Singlet | 17 | 56.984 | Doublet |
| 18 | - | - | 18 | 34.292 | Doublet |
| 19 | 1.00 | Doublet | 19 | 36.386 | Triplet |
| 20 | 1.30 | Doublet | 20 | 26.242 | Triplet |
| 21 | 1.30 | Doublet | 21 | 26.242 | Triplet |
| 22 | 1.30 | Doublet | 22 | 37.456 | Triplet |
| 23 | 1.30 | Doublet | 23 | 22.942 | Doublet |
| 24 | 1.00 | Doublet | 24 | 22.942 | Quartet |
| 25 | - | - | 25 | 22.900 | Quartet |
| 26 | 1.00 | Doublet | 26 | 19.012 | Quartet |
| 27 | 0.82 | Multiplet | 27 | 14.376 | Quartet |
| 28 | 2.00 | Singlet | 28 | 12.214 | Quartet |
| 29 | 1.00 | Doublet | 29 | 19.012 | Quartet |
| 30 | - | - | 30 | 179.794 | Singlet |
| 31 | 2.00 | Singlet | 31 | 21.311 | Quartet |

The proposed structure of tetracyclic steroid is shown below:



(iii) Effect of tetracyclic steroid on haematological parameters

The effect of tetracyclic steroid 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 0.75 mg/kg BW of tetracyclic steroid caused significant ($p < 0.05$) increase in the MCV value relative to the control.

Treatment of rats with 0.50 mg/kg BW and 0.75 mg/kg BW of tetracyclic steroid caused significant ($p < 0.05$) increase in the PCV, Hb and RBC values relative to their respective controls. Treatment of rats with 0.50 mg/kg BW and 0.75 mg/kg BW of tetracyclic steroid caused no significant ($p > 0.05$) change in the MCHC, MCH, platelet, TWBC, neutrophil, lymphocyte, eosinophil and monocyte values relative to their respective controls.

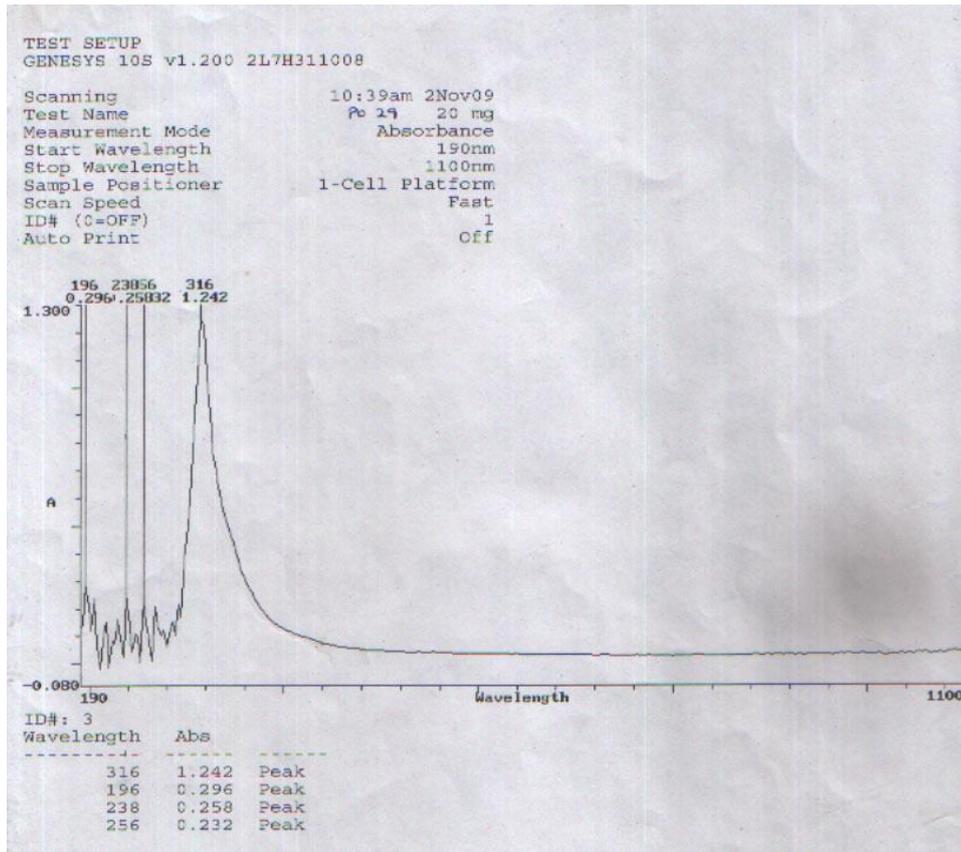


Fig. 1: UV spectrum of compound 29 (tetracyclic steroid)

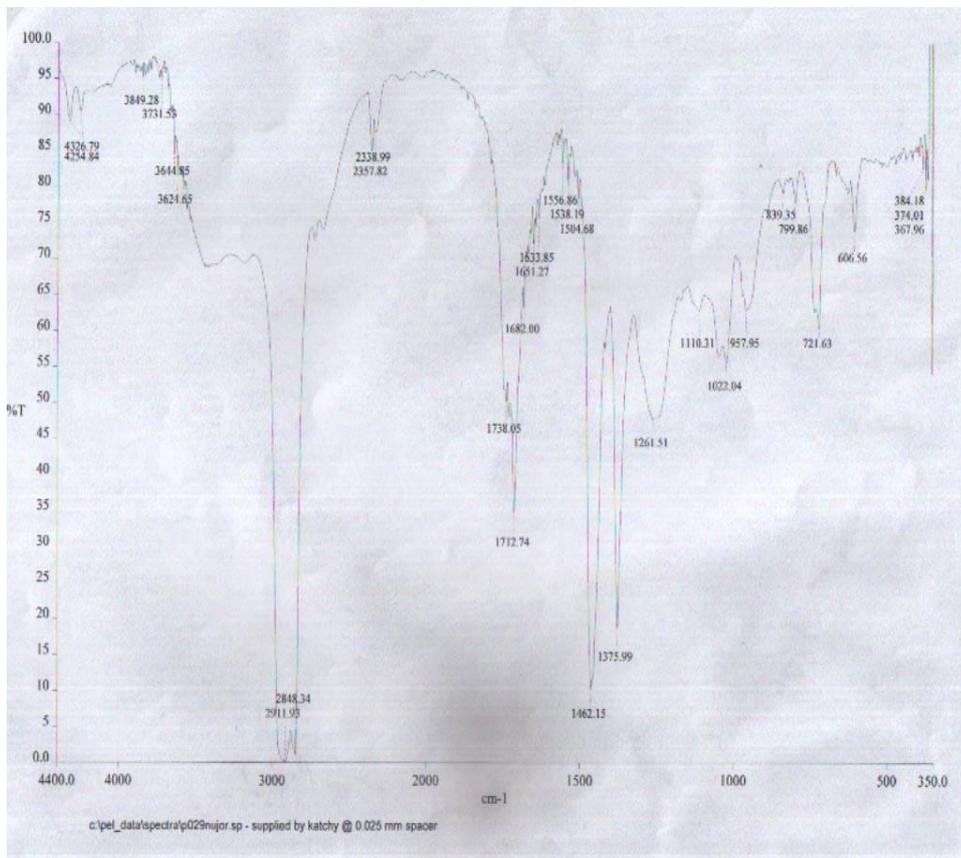


Fig. 2: IR spectrum of compound 29 (tetracyclic steroid)

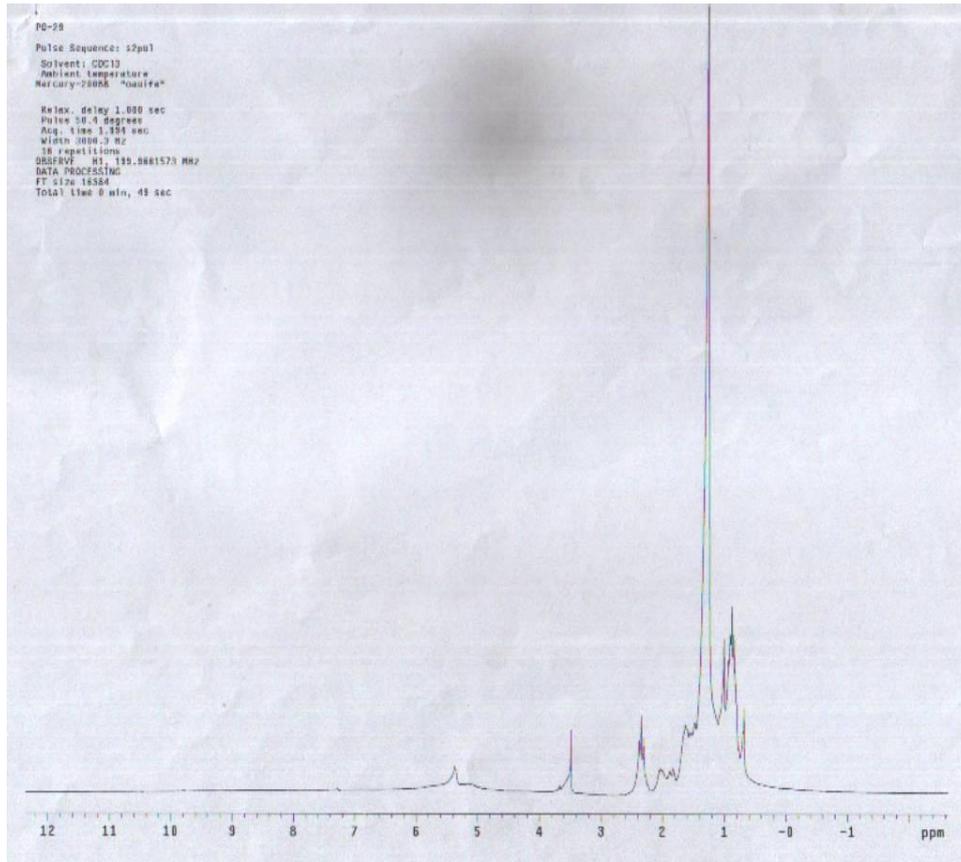


Fig. 3: $^1\text{H-NMR}$ spectrum of compound 29 (tetracyclic steroid)

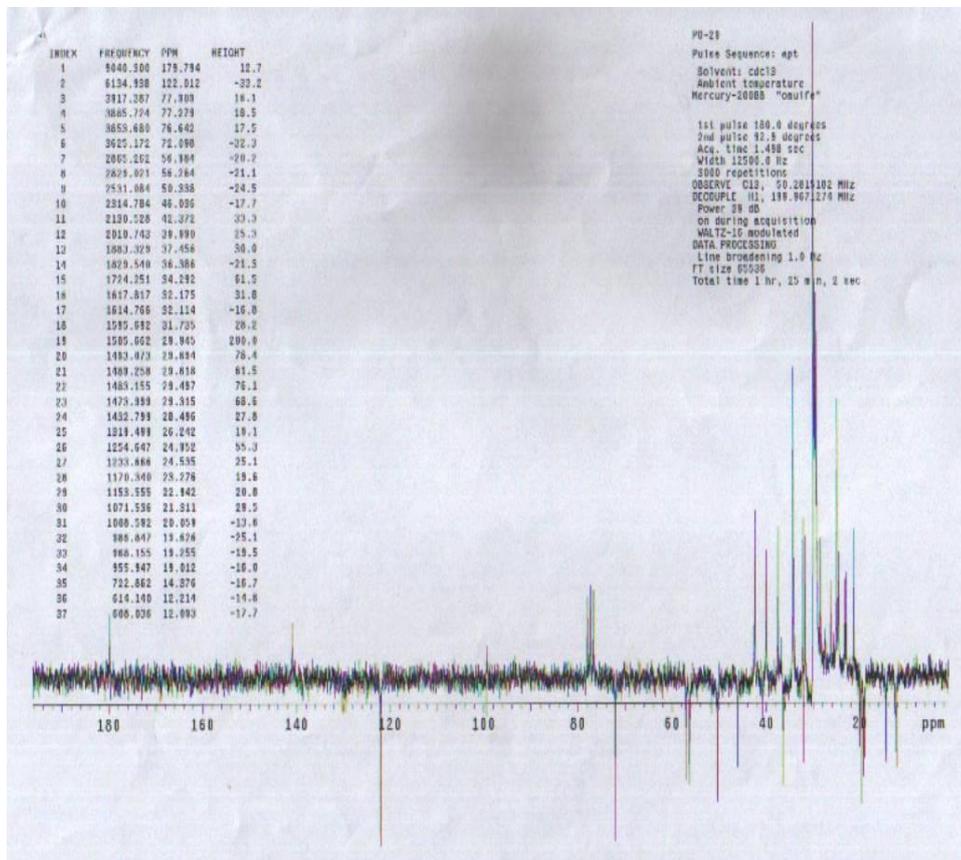


Fig. 4: $^{13}\text{C-NMR}$ of compound 29 (tetracyclic steroid)

Table 2: Effect of Tetracyclic steroid on Hematological Parameters after Treatment of Rats for 25 Days (n = 5, *P<0.05)

| Parameters | Control | 0.50 mg/kg | 0.750 mg/kg |
|---------------------------------|--------------|---------------|---------------|
| PCV (%) | 41.80 ± 1.03 | 49.30 ± 0.63* | 50.30 ± 0.48* |
| Hb (g/dl) | 13.90 ± 0.41 | 16.70 ± 0.03* | 17.10 ± 0.13* |
| RBC (x10 ⁶ /µl) | 7.07 ± 0.27 | 8.08 ± 0.18* | 8.16 ± 0.13* |
| MCV (fL) | 59.10 ± 0.78 | 61.20 ± 1.10 | 61.70 ± 0.46* |
| MCHC (g/dl) | 33.40 ± 0.25 | 33.70 ± 0.38 | 33.90 ± 0.36 |
| MCH (pg) | 19.90 ± 0.32 | 20.6 ± 0.43 | 20.9 ± 0.32 |
| TWBC (x10 ³ /µL) | 11.00 ± 0.42 | 12.00 ± 0.23 | 13.00 ± 0.20 |
| Platelets (10 ⁵ /µL) | 1.10 ± 0.10 | 1.20 ± 0.05 | 1.10 ± 0.04 |
| Neutrophils (%) | 27.00 ± 0.41 | 29.00 ± 2.16 | 26.80 ± 1.84 |
| Lymphocytes (%) | 68.00 ± 0.41 | 67.30 ± 1.70 | 68.30 ± 1.65 |
| Eosinophils (%) | 2.25 ± 0.48 | 1.50 ± 0.65 | 3.25 ± 0.25 |
| Monocytes (%) | 2.75 ± 0.63 | 2.25 ± 0.25 | 2.00 ± 0.00 |

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 tetracyclic steroid at doses of 0.50 mg/kg BW and 0.750 mg/kg BW on the haematological parameters of albino rats after treatment for 25 days is shown in Table 2.

This study has revealed that tetracyclic steroid caused increase in the PCV, Hb and RBC values. This suggests that tetracyclic steroid has the potential to stimulate erythropoietin releases 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 [15]. It could also indicate that there was an enhancement in the oxygen-carrying capacity of blood and the animal of oxygen delivered to the tissues since RBC and haemoglobin are very important in transferring respiratory gases [16]. Similar report was given by [17] in erythrocytes carrier study.

The significant increase in the MCV value caused by tetracyclic steroid may imply an induction of macrocytic anaemia, since increased MCV and MCH values are known to be indicative of macrocytic anaemia. Tetracyclic steroid caused non - significant changes in the MCV and MCH values which could be an indication of absence of macrocytic anaemia since increased MCV and MCH values are known to be indicative of macrocytic anaemia. Also tetracyclic steroid caused non- significant change in the MCHC value which suggests an absence of hereditary spherocytosis since MCHC values are known to be elevated in hereditary spherocytosis.

The insignificant change in neutrophil count caused by tetracyclic steroid 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 responses of the body has not been compromised by tetracyclic steroid; while the non- significant change in monocyte count probably indicates that the phagocytic function of the body has not been compromised by tetracyclic steroid. The non - significant change in eosinophil count probably indicates that the anti-allergic and anti-parasitic injections response of the body have not been compromised by Tetracyclic steroid.

The insignificant change in TWBC count caused by tetracyclic steroid suggests that the immune system has not been compromised. Contrary report was given by [18] in *Pelargonium reniforme* extract treated rats. Also, the insignificant change in the platelet count caused by tetracyclic steroid could be an indication that it does not have the potential to stimulate thrombopoietin production [19] with hemostatic capability of the blood maintaining the *status quo* since platelets mediate in the blood -clotting mechanism.

In conclusion, this study has shown that isolated tetracyclic steroid constituent of *Portulaca oleracea* could have some beneficial potential on the blood chemistry of albino rats. However, the effect of tetracyclic steroid on human blood chemistry is unknown,

nevertheless, considering these findings in animal model, tetracyclic steroid is thus recommended as a food supplement.

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