



Effect of fraction 4 of *Portulaca oleracea* on hematological and plasma biochemical parameters in male Wistar rats

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ABSTRACT

Portulaca oleracea is a fleshy annual herb which is distributed throughout temperate and tropical areas of world. The crude extracts of this plant have been reported to have toxic and beneficial effects on hematological functions and blood chemistry in male rats. Air-dried specimen of *Portulaca oleracea* (3.2 kg) was cold-extracted in methanol for 72 hours. The resulting methanol extract was then subjected to open column chromatography on silica gel for fractionation. Out of the 5 fractions obtained, fraction 4 was then subjected to male rats hematological and plasma biochemical bioassays. Twenty male rats (160 – 180 g) were divided into control (distilled water) and fraction 4 (1, 2, 3, mg/kg) treated groups (5 per group). The animals were orally treated on daily basis for 50 days. Red Blood Cell (RBC) and Total White Blood Cell (TWBC) counts were determined using hemocytometer. Activities of plasma Alanine Amino Transferase (ALT), Aspartate Amino Transferase (AST) and levels of total protein, globulin and albumin were determined by spectrophotometry. Treatment of rats with fraction 4 (2 mg/kg) produced significant ($p < 0.05$) reductions in PCV and lymphocyte value. Fraction 4 (1 mg/kg, 2 mg/kg, 3 mg/kg) caused significant ($p < 0.05$) increases in total protein and albumin levels relative to their respective controls. It can therefore be concluded that fraction 4 of *Portulaca oleracea* probably has both toxic and beneficial effects on hematological functions and blood chemistry in male rats.

Keywords: Fraction 4, Red blood cell, Total white blood cell, Albumin, Rats.

INTRODUCTION

The determination of levels of several constituents of blood and plasma of mammals have continued to play valuable role in the assessment of normal functioning of living organisms as changes from the normal levels have been observed with disease conditions [1].

Portulaca oleracea belongs to the family of Portulacaceae. It is a fleshy annual herb, much-branched and attaining 30 cm long. It is commonly called Purslane in English language, “Babbajibji” in Hausa language and “Esan omode or Papasan” by the Yoruba language speaking people of Nigeria [2].

It is used medicinally in Ghana for heart - palpitations [3]. The plant is used as a diuretic in Nigeria [4]. A tisane of the plant is drunk in Trinidad as a vermifuge [5]. At some areas near Benin City (Nigeria), the plant, along with other ingredients is taken as an aid to the development of the fetus [6].

It has been reported that the aqueous and methanol extracts of *Portulaca oleracea* have contractile effects on isolated intestinal smooth muscle in *in-vitro* preparations [7]. The extracts of *Portulaca oleracea* have been reported to have protective effects on hypoxic nerve tissue [8], anti-inflammatory effects [9] and wound-healing activity [10]. The skeletal muscle relaxant effect of this plant has also been reported [11].

Since this plant crude extracts have been reported to have toxic and beneficial effects on the hematological functions and blood chemistry in rats [12], this study therefore aims at investigating the effect of chromatographic fraction 4 of *Portulaca oleracea* on hematological and plasma biochemical parameters in male rats.

EXPERIMENTAL SECTION

Experimental Animals

Adult male albino rats weighing between 160 g and 180 g bred in the Pre-Clinical Animal House of the College of Medicine and Health Sciences, Afe Babalola University were used. They were housed under standard laboratory conditions and had free access to feed and water. They were acclimatized to laboratory conditions for two weeks 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 oleracea* 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 and Fractionation 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 (45 – 50 °C).

The methanol extract was then pre-absorbed with silical gel and placed in the oven at a reduced temperature (45 – 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 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)

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

Thin Layer Chromatography (TLC)

The 21 fractions were spotted on pre-coated 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).

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

Fraction 4 was then subjected to bioassay, *vis-à-vis*, its effect on hematological and plasma biochemical parameters in male rats were evaluated.

Acute Toxicity Test of Chromatographic Fraction

The acute toxicity test of chromatographic fraction 4 of *Portulaca oleracea* was evaluated in mice as described by [13]. Fifteen adult male mice weighing between 20-22 g were divided into five mice per group. Three doses of the fraction: 1 mg/kg, 5 mg/kg and 10 mg/kg were given orally to the animals. The control group mice (n=5) received 0.5 ml of distilled water. The animals were observed for seven days for behavioral changes and mortality.

Experimental Design

Twenty animals were randomly divided into four groups with each group consisting of five rats. The four groups were subjected to the following oral daily treatments for 50 days:

Group I rats received 1 mg/kg of fraction 4

Group II rats received 2 mg/kg of fraction 4

Group III rats receive 3 mg/kg of fraction 4

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

Collection of blood samples

Twenty four hours (day 51) after the last dosing of all the groups, blood samples were collected from all the animals through the medial cantus with heparinized capillary tubes into EDTA bottles for hematological and plasma biochemical analysis. Before assays, the blood was centrifuged for 5 minutes using a bench top centrifuge (Centromix) and the plasma were used for the determination of the biochemical parameters.

Determination of Hematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were determined by the Improved Neubauer haemocytometer method. The hemoglobin (Hb) concentration was determined according to [14] using the cyanomethemoglobin method. The packed cell volume (PCV) was determined by the micro - hematocrit method according to [15]. Schilling method of differential leukocyte count was used to determine the distribution of the various white blood cells [16]. Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were computed according to [14].

Determination of Plasma Biochemical Parameters

The total protein concentration was determined using the Biuret method [17] and the albumin concentration by the method of [18]. The globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration. Activities of plasma alanine transaminase (ALT) and aspartate transaminase (AST) were determined according to the method of [19]. All the above biochemical parameters were determined in the plasma using the Randox kits.

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 Duncan's Multiple Range Test. Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

The effects of different doses of fraction 4 on hematological and plasma biochemical parameters after treatment of rats for 50 days are shown in Tables 1 and 2 respectively.

Treatment of rats with fraction 4 (2 mg/kg) produced significant ($p < 0.05$) reductions in PCV, hemoglobin (Hb) and lymphocyte values relative to their respective controls. At all the treatment doses (1 mg/kg, 2 mg/kg, 3 mg/kg), fraction 4 caused no significant ($p > 0.05$) changes in RBC, MCHC and eosinophil values relative to their respective controls. Fraction 4 (1 mg/kg) caused significant ($p < 0.05$) increase in MCV and MCH values relative to their respective controls. Fraction 4 (3 mg/kg) caused significant ($p < 0.05$) reductions in TWBC and platelet values relative to their respective controls. Fraction 4 (2 mg/kg) caused significant ($p < 0.05$) reductions in lymphocyte and monocyte values relative to their respective controls. Fraction 4 (2 mg/kg) produced significant ($p < 0.05$) increase in neutrophil value relative to the control.

Treatment of rats with fraction 4 (1 mg/kg, 2 mg/kg, 3 mg/kg) caused significant ($p < 0.05$) increase in total protein and albumin levels relative to their respective controls. Fraction 4 (1 mg/kg, 3 mg/kg) produced significant ($p < 0.05$) increase in globulin level relative to the control. Fraction 4 (1 mg/kg, 2 mg/kg, 3 mg/kg) produced significant ($p < 0.05$) increase in the activities of AST and ALT relative to their respective controls.

Table 1: Effects of Different Doses of Fraction 4 on Hematological Parameters after Treatment of Rats for 50 Days

Parameters	Control	1 mg/kg	2 mg/kg	3 mg/kg
PCV (%)	41.80 ± 2.32	42.00 ± 1.41	38.30 ± 0.63*	42.30 ± 0.48
Hb (g/dl)	13.10 ± 0.83	13.50 ± 0.54	11.80 ± 0.28*	13.60 ± 0.13
RBC ($\times 10^6/\mu\text{l}$)	7.04 ± 0.39	6.70 ± 0.16	6.64 ± 0.31	7.03 ± 0.07
MCV (fL)	59.20 ± 0.34	62.60 ± 0.57*	58.10 ± 1.70	60.20 ± 0.08
MCHC (g/dl)	31.30 ± 0.27	32.00 ± 0.23	30.80 ± 0.31	32.10 ± 0.07
MCH (pg)	18.60 ± 0.26	20.10 ± 0.32*	17.90 ± 0.37	19.30 ± 0.05
TWBC ($\times 10^3/\mu\text{L}$)	8.34 ± 0.62	6.40 ± 0.32	7.49 ± 0.76	5.00 ± 0.54*
Platelets ($10^5/\mu\text{L}$)	1.20 ± 0.13	0.76 ± 0.07*	0.99 ± 0.07	0.82 ± 0.02*
Neutrophils (%)	32.80 ± 1.93	39.30 ± 4.11	46.30 ± 4.84*	30.30 ± 1.25
Lymphocytes (%)	64.00 ± 1.41	58.30 ± 3.92	51.00 ± 4.60*	68.00 ± 1.08
Eosinophils (%)	1.75 ± 0.48	1.00 ± 0.41	2.00 ± 0.41	0.75 ± 0.25
Monocytes (%)	2.00 ± 0.41	1.25 ± 0.48	0.75 ± 0.25*	1.00 ± 0.41

($n = 5$, * $p < 0.05$).

Table 2: Effects of Varying Doses of Fraction 4 on Serum Biochemical Parameters after Treatment of Rats for 50 Days

Parameters	Control	1 mg/kg	2 mg/kg	3 mg/kg
Total Protein (gm%)	4.90 ± 0.18	6.08 ± 0.05*	5.73 ± 0.12*	6.03 ± 0.09*
Albumin (gm%)	1.28 ± 0.10	1.60 ± 0.08*	1.58 ± 0.13*	1.70 ± 0.11*
Globulin (gm%)	3.63 ± 0.10	4.48 ± 0.12*	4.18 ± 0.05	4.73 ± 0.31*
AST (μL)	19.30 ± 2.32	30.80 ± 1.44*	27.80 ± 2.63*	32.30 ± 0.48*
ALT (μL)	14.80 ± 1.84	23.30 ± 1.03*	23.00 ± 2.16*	27.30 ± 1.55*

($n = 5$, * $p < 0.05$).

It was observed that the highest dose of fraction 4 caused no mortality or behavioral changes in all the treated animals which indicates that fraction 4 has wide safety margin.

The results have shown that fraction 4 caused no significant changes on the RBC. This could indicate that fraction 4 does not have the potential to stimulate erythropoietin release from the kidneys which is the humoral regulator of RBC production [20]. It could also indicate that there were no changes in the oxygen carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and hemoglobin (Hb) are very important in transferring respiratory gases [21]. It has been reported that values of RBC and associated parameters lower than normal ranges are indicative of anemic conditions while higher values are suggestive of polycythemia [22]; thus, the fraction may not have the potential to induce anemia or polycythemia. Also, the fraction may not have adverse effects on the bone marrow, kidney and hemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and hemoglobin metabolism [23]. The fraction caused significant decrease in the PCV values which could indicate an induction of anemia [22]. Similar result was reported by [24] in aspirin treated rats. Fraction 4 caused significant increase in hemoglobin concentration which probably indicates an enhancement in the oxygen –

carrying capacity of the blood. Similar result was reported by [25] in *Corchoru olitorius* extract treated rats. Fraction 4 caused significant increase in MCV and MCH values which probably indicate the induction of macrocytic anemia, since increased MCV and MCH values are known to be indicative of macrocytic anemia [26]. Similar result was reported by [27] in *Azadirachta indica* extract treated chicks. Fraction 4 caused significant decrease in TWBC values which probably indicates a reduction in the ability of the body to defend against invading organisms [28]. Similar result was reported by [29] in *Viscum album* extract treated rats. The fraction caused significant decrease in the platelet value which probably indicates a compromise on the hemostatic function of the body. Similar result was reported by [30] in *Fadogia agrestis* extract treated rats. The fraction caused significant decrease in lymphocyte value which probably indicates a compromise in the acquired immune response of the body. Similar result was reported by [31] in isolated ergosterol treated rats. The fraction caused significant increase in neutrophil count which probably indicates an enhancement on the ability of the body to attack and destroy invading bacteria, viruses and other injurious agents (phagocytosis). Similar result was reported by [32] in *Denmettia tripetala* extract treated rats. The fraction caused significant decrease in the monocyte value which probably indicates that the phagocytic function of the body could be compromised [33]. Contrary result was reported by [34] in *Saccharomyces cerevisiae* extract fed hens. The fraction caused no significant change in eosinophil value which could indicate that it has no effect on the anti - allergic and anti - parasitic infectious responses of the body. Contrary result was reported by [35] in *Arctotis actotoides* extract treated rats and mice.

The results of the plasma biochemical study have shown that treatment of rats with fraction 4 caused significant increase in total protein level. This could indicate an enhancement in the buffering capacity of the blood as well as an increase in colloid osmotic pressure which could prevent loss of fluid from the capillaries, since plasma proteins have been reported to be responsible for 15 % of buffering capacity of blood [24] and that osmotic pressure caused by the plasma proteins (called colloid osmotic pressure) tends to cause fluid movement by osmosis. Similar result was reported by [36] in *Euphorbia heterophylla* extract treated rats. The fraction caused significant increase in albumin level which probably indicates an enhancement in the plasma levels of metals, ions, fatty acids, amino acids, bilirubin and enzymes; since it has been reported that albumin serves as a carrier for metals, ions, fatty acids, amino acids, bilirubin, enzymes and drugs [24]. Similar result was reported by [37] in *Enicostem maaxillare* extract treated rats. The fraction produced significant increase in globulin level which probably indicates an enhancement in both the natural and acquired immunity of the body against invading organisms, since it has been reported that globulins are principally responsible for the body's both natural and acquired immunity against invading organisms [33]. Contrary result was reported by [12] in *Portulaca oleracea* extracts treated rats. The significant increase in the activity of AST induced by fraction 4 probably indicates an induction of tissue necrosis, since it has been reported that elevation in the activity of AST can be associated with cell necrosis of many tissues, which allows leakage of large amounts of this enzyme into the blood [38]. Similar result was reported by [39] in *Sida rhombifolia* extract treated mice and rats. The fraction caused a significant increase in the activity of ALT which probably indicates an induction of hepatic damage, since it has been reported that ALT is present in the liver and other cells and is particularly useful in measuring hepatic necrosis, especially in small animals [19]. Similar result was reported by [40] in *Moringa oleifera* extract treated rats.

CONCLUSION

In conclusion, this study has shown that chromatographic fraction 4 of *Portulaca oleracea* has toxic effect and beneficial potentialities on the hematological functions and blood chemistry of male rats. However, the effect of fraction 4 of this plant on human hematological functions and blood chemistry are unknown; nevertheless, considering these findings in animal model, it is recommended that moderation should be exercised in the consumption of *Portulaca oleracea*.

Acknowledgement

I want to sincerely thank Aunty Josephine of the Department of Veterinary Pathology, Faculty of Veterinary Medicine, University of Ibadan, for her technical assistance.

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