

Effect Of Chromatographic Fraction of *Portulaca Oleracea* on Haematological and Plasma Biochemical Parameters In Male Albino Rats

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Abstract: The effect of oral administration of chromatographic fraction of *Portulaca oleracea* at doses of 1 mg/kg BW, 2 mg/kg BW and 3 mg/kg BW on haematological and plasma biochemical parameters of albino rats were investigated. The fraction was administered on daily basis for 30 days and blood samples were collected for analyses.

Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction caused significant ($p < 0.05$) increases in TWBC and lymphocyte counts respectively relative to their controls, but treatment of rats with 2 mg/kg BW of fraction caused significant ($p < 0.05$) decrease in monocyte count relative to the control.

Treatment of rats with all the treatment doses of fraction (1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW) caused significant ($p < 0.05$) increments in total protein and globulin levels relative to their respective controls, also treatment of rats with all the treatment doses of fraction caused significant ($p < 0.05$) increases in the activities of AST and ALT relative to their respective controls.

These findings on haematological and plasma biochemical parameters suggest that the possible changes in blood chemistry of the treated rats were due to the chromatographic fraction of *Portulaca oleracea*.

Key words: *Portulaca oleracea*, Chromatographic fraction, Red blood cell, Albumin, Albino rats.

I. 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 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 (Oyededeji *et al*, 2007).

It has also been reported that aqueous and methanolic extracts of *Portulaca oleracea* have some toxic and beneficial potentials on the blood chemistry of albino rats (Oyededeji and Bolarinwa, 2012).

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 chromatographic fraction of *Portulaca oleracea* on the haematological and plasma biochemical parameters in male albino rats.

II. Materials and Methods

Experimental Animals

Adult male albino rats weighing between 160 g and 180 g bred in the Animal House of Physiology Department, LAUTECH, Ogbomosho were used. They were housed under standard laboratory conditions with a 12 hours daylight cycle 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 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 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 (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).

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 analyses of all the fractions indicated fraction 2 as the fraction that contained many components and this fraction was subjected to bioassay, *vis-à-vis*, its effect on the haematological and plasma biochemical parameters of male albino rats were evaluated.

The dosages of the fraction administered in this study were extrapolated from that reported by Miladi-Gorgi *et al.*, (2004).

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 30 days:

Group I rats received 1 mg/kg BW of fraction

Group II rats received 2 mg/kg BW of fraction

Group III rats receive 3 mg/kg BW of fraction

Group IV 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 and plasma biochemical studies. Before assays, the blood samples were centrifuge for 5 minutes using a bench-top centrifuge (Centromix) and the supernant plasma was then used for the determinations of the biochemical parameters.

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 leucocyte 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).

Determination of Plasma Biochemical Parameters

The total protein concentration was determined using the Biuret method (Reinhold, 1953) and the albumin concentration by the method of Doumas *et al.* (1971). 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 Duncan (1994). 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 $p < 0.05$.

III. Result

The effects of various doses of fraction on haematological and plasma biochemical parameters of albino rats after treatment of rats for 30 days are shown respectively in Tables 1 and 2.

Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction caused significant ($p < 0.05$) increases in TWBC and lymphocyte counts respectively relative to their controls, but treatment of rats with 2 mg/kg BW of fraction caused significant ($p < 0.05$) decrease in monocyte count relative to the control. Treatment of rats with all the doses of fraction caused insignificant ($p > 0.05$) changes in PCV, Hb, RBC, platelet, neutrophil, eosinophil and hematometric indices (MCV, MCHC, MCH) values relative to their respective controls.

Treatment of rats with all the treatment doses of fraction (1 mg/kg BW, 2 mg/kg BW, 3 mg/kg BW) caused significant ($p < 0.05$) increases in total protein and globulin levels relative to their respective controls. Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction caused significant increases in albumin levels relative to the control. Treatment of rats with all the treatment doses of fraction caused significant ($p < 0.05$) increases in the activities of AST and ALT relative to their respective controls.

IV. Discussion

The values obtained for RBC showed the non-significant effects of 30-day treatment of rats with fraction on red blood cells (RBC) counts and indices relating to it (Hb, PCV, MCV, MCH and MCHC) when compared with the control. This is an indication that there was no destruction of red blood cells and no change in the rate of production of RBC (erythropoiesis). This also shows that fraction does not have the potential to stimulate erythropoietin release from the kidneys, which is the humoral regulator of RBC production (Polenakovic and Sikole, 1996). The non-significant effects of treatment of rats with fraction at all doses also indicate that there were no change in the oxygen-carrying capacity of the blood and the amount of oxygen delivered to the tissues since RBC and haemoglobin (Hb) are very important in transferring respiratory gases (De Gruchy, 1976). 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 (American Diabetes Association, 2000), thus, the treatment of rats with fraction may not have the potential to induce anemia or polycythemia. Also, the treatment of rats with fraction at all treatment doses may not have adverse effects on the bone marrow, kidney and haemoglobin 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 haemoglobin metabolism (Young and Maciejewski, 1997).

The fraction 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, fraction 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 fraction 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 significant increase in lymphocyte count suggests that the acquired immune responses of the body has been enhanced by the fraction; while the significant decrease in monocyte count probably indicates that the phagocytic function of the body has been compromised by fraction. The non – significant change in eosinophil count probably indicates that the anti-allergic and anti-parasitic infectious responses of the blood have not been compromised by fraction.

The significant increase in TWBC count caused by fraction suggests that the immune system has been enhanced. Similar report was given by Adewusi and Afolayan (2009) in *Pelargonium reniforme* extract treated rats. Also, the insignificant change in the platelet count caused by fraction could be an indication that it does not has the potential to stimulate thrombopoietin production (Li *et al.*, 1999) with the hemostatic capability of the blood maintaining the *status quo* since platelets mediate in the blood –clotting mechanism.

Treatment of rats with fraction caused significant increases in total protein levels, which probably indicates that the buffering capacity of the blood and body fluid balance have been enhanced. Similar report was given by Adewusi and Afolayan (2009) in *Pelargonium reniforme* extract treated rats. The fraction caused significant increase in albumin level which suggests an enhancement in plasma level of metals, ions, fatty acids, amino

acids, bilirubin and enzymes. Similar report was given by Adedapo *et. al.*, (2007) in rats treated with *P. amarus* and *C. anontifolius* extracts. The fraction caused significant increase in globulin level which probably indicates an enhancement in both the natural and acquired immunity of the body against invading organisms. The fraction caused significant increase in the activity of ALT which probably indicates the hepato-toxic potential of the fraction. The fraction also caused significant increase in the activity of AST which could indicate induction of tissue necrosis.

In conclusion, this study has shown that chromatographic fraction of *Portulaca oleracea* could have some toxic and beneficial potentialities on the blood chemistry of albino rats. However, its effect 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 *Portulaca oleracea*.

Table 1: Effect of Fraction on Haematological Parameters after Treatment of Rats for 30 Days (n = 5, *p < 0.05)

Parameters	Control	1mg/kg	2mg/kg	3mg/kg
PCV (%)	41.80 ± 2.32	42.80 ± 1.03	41.00 ± 1.47	39.00 ± 1.08
Hb (g/dl)	13.10 ± 0.83	13.60 ± 0.43	13.30 ± 0.31	12.70 ± 0.12
RBC (x10 ⁶ /μl)	7.04 ± 0.39	7.14 ± 0.15	7.05 ± 0.22	6.58 ± 0.08
MCV (FL)	59.20 ± 0.34	59.80 ± 0.68	58.10 ± 0.39	59.2 ± 0.97
MCHC (g/dl)	31.30 ± 0.27	31.90 ± 0.25	32.40 ± 0.44	32.7 ± 1.03
MCH (pg)	18.60 ± 0.26	19.10 ± 0.31	18.80 ± 0.16	19.30 ± 0.31
TWBC (x10 ³ /μL)	8.34 ± 0.62	11.00 ± 1.02*	6.30 ± 0.51	8.10 ± 0.69
Platelets (10 ⁵ /μL)	1.20 ± 0.13	1.40 ± 0.12	1.10 ± 0.09	1.20 ± 0.08
Neutrophils (%)	32.80 ± 1.93	35.80 ± 1.44	26.80 ± 3.92	36.80 ± 3.40
Lymphocytes (%)	64.00 ± 1.41	62.80 ± 1.31	77.80 ± 4.09*	61.00 ± 3.34
Eosinophils (%)	1.75 ± 0.48	0.75 ± 0.25	1.25 ± 0.25	1.00 ± 0.41
Monocytes (%)	2.00 ± 0.41	1.00 ± 0.41	0.25 ± 0.25*	1.25 ± 0.63

Table 2: Effect of Fraction on Serum Biochemical Parameters after Treatment of Rats for 30 Days (n = 5, *p<0.05)

Parameters	Control	1mg/kg	2mg/kg	3mg/kg
Total Protein (gm%)	4.90 ± 0.18	5.80 ± 0.27*	6.43 ± 0.23*	6.28 ± 0.24*
Albumin (gm%)	1.28 ± 0.10	1.60 ± 0.08*	1.60 ± 0.08*	1.48 ± 0.06
Globulin (gm%)	3.63 ± 0.10	4.53 ± 0.09*	4.73 ± 0.12*	5.00 ± 0.14*
AST (μ/L)	19.30 ± 2.32	30.30 ± 2.50*	29.30 ± 1.84*	39.80 ± 1.25*
ALT (μ/L)	14.80 ± 1.84	27.8 ± 2.06*	23.00 ± 1.63*	31.80 ± 1.44*

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