Research Article

Effect of Fraction 5 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 5 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 3 mg/kg BW of fraction 5 caused significant ($p<0.05$) decrease in the PCV and RBC values relative to their respective controls. Treatment of rats with 1 mg/kg BW of fraction 5 resulted in significant ($p<0.05$) decrease in Hb and MCHC values relative to their respective controls, while treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) decrease in monocyte counts relative to the control. Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) increases respectively in total protein level and ALT activity relative to their respective controls. Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) increase in albumin level and AST activity 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 chromatographic fraction 5 of *Portulaca oleracea.*

Keywords: Albino rats, Albumin, Chromatographic fraction 5, *Portulaca oleracea*, Red blood cell.

INTRODUCTION

Portulaca oleracea belongs to the family of *Portulacacea*. 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.[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 foetus.[5] It has been reported that the crude extracts of *Portulaca oleracea* have no deleterious effects on the reproductive functions of female albino rats.[6] It has been reported that isolated ergosterol constituent of *Portulaca oleracea* has deleterious effect on reproductive functions in male albino rats.[7] It has also been reported that isolated tetracyclic steroid constituent of *Portulaca oleracea* has deleterious effect on the reproductive functions in male albino rats.[8]

This study aims at investigating the effect of chromatographic fraction 5 of *Portulaca oleracea* on the haematological and plasma biochemical parameters in male albino rats.

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, Ogbomoso 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 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 (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 (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).

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).

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

Fraction 5 was then subjected to bioassay, vis-à-vis, its effect on haematological and plasma biochemical parameters in male albino rats were evaluated.

The dosages of the fraction administered in this study were extrapolated from that reported by.[9]

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 5

Group II rats received 2 mg/kg BW of fraction 5

Group III rats receive 3 mg/kg BW of fraction 5

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[10], using the cyanomethaemoglobin method. The packed cell volume (PCV) was determined by the micro-haematocrit method according to.[11] Schilling method of differential lecukocyte count was used to determine the distribution of the various white blood cells.[12] Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were computed according to.[10]

Determination of Plasma Biochemical Parameters

The total protein concentration was determined using the Biuret method[13] and the albumin concentration by the method of.[14] 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.[15] 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

The effects of various doses of fraction 5 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 3 mg/kg BW of fraction 5 caused significant ($p<0.05$) decrease in PCV and RBC values relative to their respective controls, while treatment of rats with 1 mg/kg BW of fraction 5 resulted in significant ($p<0.05$) decrease in Hb and MCHC values relative to their respective controls. Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) decrease and increase respectively in MCH value relative to the control, while treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) decrease in monocyte counts relative to the control. Treatment of rats with 1 mg/kg BW, 2 mg/kg BW and 3 mg/kg BW of fraction 5 caused no significant ($p>0.05$) changes in MCV, platelet, TWBC, neutrophil, lymphocyte and eosinophil values relative to their respective controls.

Treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) increases respectively in total protein level and ALT activity relative to their respective controls, while treatment of rats with 1 mg/kg BW and 2 mg/kg BW of fraction 5 caused significant ($p<0.05$) increases in albumin level and AST activity relative to their respective controls. Treatment of rats with 1 mg/kg BW, 2 mg/kg BW and 3 mg/kg BW of fraction 5 caused no significant ($p>0.05$) changes in globulin level relative to the control.

Table 1: Effect of Varying Doses of Fraction 5 on Haematological Parameters after Treatment of Rats for 30 Days

Parameters	Control	1 mg/kg	2 mg/kg	3 mg/kg
PCV (%)	41.80 ± 2.32	39.30 ± 0.25	40.80 ± 2.25	38.00 ± 0.41*
Hb (g/dl)	13.10 ± 0.83	11.60 ± 0.64*	13.30 ± 0.84	12.00 ± 0.26
RBC (x10^6/µl)	7.04 ± 0.39	6.69 ± 0.11	6.61 ± 0.39	6.37 ±0.04*
MCV (FL)	59.20 ± 0.34	58.80 ± 0.71	61.60 ± 0.29	59.60 ± 0.84
MCHC (g/dl)	31.30 ± 0.27	29.50 ± 1.56*	32.70 ±0.29	31.70 ± 0.43
MCH (pg)	18.60 ± 0.26	17.40 ± 1.06*	20.10 ± 0.15*	18.90 ± 0.39
TWBC (x10^3/µL)	8.34 ± 0.62	8.05 ±0.41	6.76 ± 0.88	9.14 ± 1.12
Platelets (10^5/µL)	1.20 ± 0.13	1.10 ± 0.06	0.98 ± 0.10	1.50 ± 0.26
Neutrophils (%)	32.80 ± 1.93	38.00 ± 7.26	25.00 ± 3.49	26.30 ± 1.25
Lymphocytes (%)	64.00 ± 1.41	60.00 ± 7.56	73.80 ± 3.07	72.00 ± 2.04
Eosinophils (%)	1.75 ± 0.48	1.25 ± 0.25	1.00 ±0.41	0.25 ± 0.25
Monocytes (%)	2.00 ± 0.41	0.75 ± 0.25*	0.25 ± 0.25*	1.25 ± 0.63

(n = 5, *p< 0.05)

Table 2: Effect of Varying Doses of Fraction 5 on Serum Biochemical Parameters after Treatment of Rats for 30 Days

Parameters	Control	1 mg/kg	2 mg/kg	3 mg/kg
Total Protein (gm%)	4.90 ± 0.18	6.00 ± 0.36*	4.83 ±0.52	5.18 ± 0.91
Albumin (gm%)	1.28 ± 0.10	1.78 ± 0.06*	1.90 ± 0.08*	1.40 ± 0.11
Globulin (gm%)	3.63 ± 0.10	4.23 ± 0.37	3.33 ± 0.47	3.78 ± 0.10
AST (µ/L)	19.30 ± 2.32	24.80 ± 1.31*	27.00 ± 2.94*	24.30 ± 1.70
ALT (µ/L)	14.80 ± 1.84	18.80 ± 2.02	23.80 ± 2.50*	21.00 ± 0.82*

(n = 5, *p<0.05)

DISCUSSION

Fraction 5 caused significant decrease in PCV value, this could indicate an induction of anaemia.[16] Similar results were reported by[17] in fractions 3 and 4 of *Cnestis ferruginea* treated rats. Fraction 5 caused significant reductions in RBC counts, which might also indicate the induction of anaemia. Similar results were reported by[18] in *A. Cordifolia, P. amarus, P. muellerianus and S. virosa* extracts treated rats. Fraction 5 caused significant increase in MCH values which probably indicates the induction of macrocytic anaemia, since increased MCV and MCH valves are known to be indicative of macrocytic anaemia.[19] Similar result was reported by[20] in *Azadirachta indica* extract treated chicks.

Fraction 5 caused increase in the TWBC value, this probably indicates an enhancement in the ability of the body to defend against invading organisms.[21] Similar result was report by[22] in *viscum album* extract treated rats. Fraction 5 caused significant decrease in monocyte values which indicates that the phagocytotic function of the body could be compromised.[23] Similar result was reported by[24] in Neem extract fed chickens. Fraction 5 caused increase in platelet value which probably indicates an enhancement in the hemostatic function of the body. Similar result was reported by[25] in *Fadogia agrestis* extract treated rats.

Fraction 5 caused significant increase in total protein level which might indicate an enhancement in the buffering capacity of blood and colloid osmotic pressure. Similar result was reported by[26] in *Pelargonium reniforme* extract treated rats. The significant increase in albumin level caused by fraction 5 could indicate an increase in serum 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, fatly acids, amino acids, bilirubin, enzymes and drugs.[21] Similar result was reported by[27] in *Enicostemma axillare* extract treated rats.

Fraction 5 caused 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.[15] Similar result was reported by[28] in *Phyllantus amarus* extract treated rats. The significant increase in AST activity caused by fraction 5 could indicate 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, pathology involving the skeletal or cardiac muscle and/or hepatic parenchyma, allows leakage of large amounts of this enzyme into the blood.[29] Contrary result was reported by[30] in *Murraya koenigii* extracts treated rats.

CONCLUSION

In conclusion, this study has shown that chromatographic fraction 5 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.*

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