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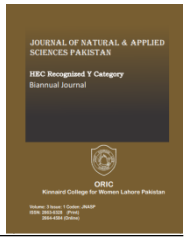

SUB-CHRONIC EFFECT OF METHANOL STEM EXTRACT OF PORTULACA OLERACEA ON HEMATOLOGICAL PARAMETERS IN MALE ALBINO RATS.

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Abstract

Portulaca oleracea linn is widely used worldwide as both a culinary vegetable and a medicinal plant. This research was conducted to explore the sub-chronic impact of orally administering methanol stem extracts of Portulaca oleracea on hematological parameters in male albino rats. The motivation for this investigation was the lack of knowledge on the toxicity of Portulaca oleracea in long-term usage. A total of sixty-four animals were allocated into four groups, with each group consisting of sixteen rats selected at random. Group 1 (Control) was given 0.5 ml of distilled water (vehicle), whereas Groups 2, 3, and 4 were given 125, 250, and 500 mg/kg bw of the extract, respectively, for a duration of 60 days by oral gavage. On days 15, 30, 45, and 60, four (4) rats from each group were anesthetized, and a blood sample was obtained for hematological analysis. Group 2 (125mg/kg) saw a statistically significant drop ($p<0.05$) in haematocrit level on day 15, followed by a statistically significant rise ($p<0.05$) on day 45. The white blood cell count showed a notable rise after 15 days and 60 days for group 2 and 4, respectively. There was no noticeable change in the levels of hemoglobin and red blood cells in any of the groups for the whole 60-day treatment period. The platelet count showed a substantial increase. This result indicates that there are no negative changes in the blood composition linked with the use of the extract in phytotherapy. Therefore, it can be concluded that the extract is non-toxic to animals and, by extension, to people.

Keywords:
Hematological, rats, blood, vegetable, toxic

1.Introduction

Traditional medicinal plants often include bioactive chemicals that may have potential harm when utilized for treating diseases. While medicinal herbs are often believed to be harmless due to their long history of usage, there is little research about their systemic toxicity (Kuete, 2014). Many medicinal plants include phytochemicals including alkaloids, glycosides, and terpenoids. These compounds may have a beneficial influence on health, either by acting individually or by working together (Nasri & Shirzad, 2013). *Portulaca oleracea* is a succulent plant that belongs to the Portulacaceae family. It is an annual plant and is popularly known as purslane or pigweed in English (Lanska, 1992). The substance has a wide range of effects on the body, including its ability to kill bacteria, prevent ulcers, reduce inflammation, protect against damage caused by harmful molecules, promote healing of wounds, induce bowel movements, strengthen the heart, soften the skin, relax muscles, increase urine production, and treat osteoporosis and psoriasis. Purslane has been shown to have superior nutritional quality compared to commonly produced vegetables, with higher levels of β-carotene, ascorbic acid, alpha-linolenic acid (ALA), and antioxidant characteristics (Liu *et al.,* 2000). Although *Portulaca oleracea* has several applications and contains diverse chemical ingredients, there is a lack of information on the sub-chronic toxicity of the plant in the existing literature, perhaps due to its extensive usage. This prompted the study, which aims to determine the safety of *Portulaca oleracea* in relation to its many use in traditional medicine, as a food source for people, and as animal feed. The research aims to focus its investigation on the haematological indicators in order to narrow down its toxicity analysis.

2. Materials and Methods

2.1 Collection of Plant Material and Authentication

Portulaca oleracea linn. Stems were freshly harvested from the Alakahia area of Port Harcourt, Nigeria, between January 2021 and February 2021. Dr. Chimezie Okeke from the Department of Plant Science and Biotechnology at the University of Port Harcourt in Rivers State, Nigeria, identified the plant. A sample of the plant was then stored at the University of Port Harcourt Herbarium with the identification number UPH/V/1302.

2.2 Preparation of Plant Extract

The plant was collected and then the stems were dried in the shade at room temperature until they reached a steady weight, which took six weeks. The desiccated stems of Portulaca oleracea were measured and pulverized into a fine powder. The process of cold maceration was used to extract the solvent successively over a duration of 72 hours, following the methodology outlined by Harborne (1998). Analytical grade methanol was used for this purpose. A 500 g quantity of the powdered stems of P. oleracea was soaked in 1.5 Liters of methanol for 72 hours, with the solvent being replaced every 24 hours. The filtrate produced from filtering using Whatman's No. 1 filter paper was concentrated using a rotary evaporator (Model

No: RE-52A) under vacuum at a temperature of 45°C. It was then transferred to an evaporating dish and dried over a water bath (Digital thermostatic water bath, Jinotech instruments) at the same temperature. The methanol stem extract of Portulaca oleracea (MSEPO) was dried and kept in a desiccator.

2.3 Animals

The research used healthy Wistar rats of both sexes, weighing between 135 and 150 grams, which were locally raised at the animal house of the Department of Pharmacology, Faculty of Basic Clinical Sciences, UNIPORT, Port Harcourt. The animals were kept in conventional enclosures for duration of two (2) weeks before the commencement of the study, in order to facilitate their adaptation to the habitat, which was maintained under natural atmospheric conditions. The animals were provided with unlimited access to regular laboratory chow (Top Feed) and water.

2.4 Experimental Design

After the animals had become used to their environment, they were randomly divided into four groups, each containing sixteen individuals, for the following treatments: Group A (Control) was administered 0.5 ml/kg body weight of 20% distilled water (vehicle), Group B was administered 125 mg/kg body weight of extract, Group C was administered 250 mg/kg body weight of extract, and Group D was administered 500 mg/kg body weight of extract. The extract and vehicle were administered orally by gavage on a daily basis for a period of 60 days. Weighing of the animals was conducted on a weekly basis, and the dosage was modified appropriately. On days 15, 30, 45, and 60, four rats from each group were anesthetized and blood samples were obtained by puncturing the heart and collecting them in bottles containing Ethylenediaminetetraacetic acid (EDTA). The blood samples that were gathered were used to determine several haematological parameters, including haematocrit, haemoglobin concentration, erythrocyte count, leucocyte count, platelet count, and differential cell count, using the methodology outlined by Cheesbrough in 2006. The erythrocyte, leucocyte, and platelet counts were measured using the enhanced Neubauer haemocytometer technique. The concentration of haemoglobin (Hb) was measured using the cyanomethaemoglobin method. The haematocrit was assessed using the microhaematocrit technique. The differential leukocyte count was used to ascertain the distribution of the different types of white blood cells in the bloodstream.

2.5 Ethical Approval

The research procedures received official approval from the Research Ethics Committee of the Centre for Research Management and Development, University of Port Harcourt, under the reference number UPH/CEREMAD/REC/MM75/074. The rats used in the study were treated with compassion and in compliance with the ethical guidelines and regulations governing the use of animals in research, as authorized by the University.

2.6 Statistical Analysis

The statistical analysis was conducted using SPSS 21. The results were presented as the mean ±

standard error of the mean (SEM) and the data were analyzed using a one-way analysis of variance (ANOVA) followed by the Tukey post-hoc test. The significance threshold was established at a p-value of less than 0.05.

3. Results

3.1 Acute Toxicity Testing and Dose calculation

The acute toxicity test performed by Obinna *et al.,* 2021 on the plant extract did not reveal any instances of death, illness, or observable indications of toxicity. The dosages administered in this trial were 1/40th, 1/20th, and 1/10th of the highest dose, which was 5000 mg/kg. As a result, the research used dosages of 125, 250, and 500 mg/kg.

3.2 Effect of methanol stem extract of Portulaca oleracea on haematological indices

The significant haematological changes after administration when compared with control, were observed in haematocrit (decrease ($p<0.05$) in day 15 and increase ($p<0.05$) in day 45 for 125mg/kg dose), WBC (significant increase ($p<0.05$) in day 15 for 125mg/kg and day 60 for 125mg/kg and 500 mg/kg respectively), platelet (significant decrease ($p<0.05$) in day 15 for 125 mg/kg and significant increase ($p<0.05$) in day 15, 30 and 60 for 500 mg/kg), Neutrophil (Significant increase ($p<0.05$) in day 15 and 60 for 125 mg/kg and 500 mg/kg doses respectively), Lymphocyte (significant increase ($p<0.05$) in day 15 and 60 for 125 mg/kg and significant increase ($p<0.05$) for 500 mg/kg), Midsized cells (Monocytes) (significant increase ($p<0.05$) in day 15 for 125mg/kg and 500mg/kg respectively with a significant increase ($p<0.05$) in day 60 for 500mg/kg), Mean Corpuscular Volume (significant decrease ($p<0.05$) day 60 for 125mg/kg and 500mg/kg dose respectively), Mean Cell Hemoglobin (significant increase ($p<0.05$) in day 30 for 250mg/kg), Mean Cell Hemoglobin Concentration (significant increase ($p<0.05$) in day 30 for 125, 250, and 500mg/kg dose respectively)

Table 1: Effect of different doses of MSEPO on Haematocrit and haemoglobin level

Parameter	Duration							
	Haematocrit (%)				Haemoglobin (g/dl)			
Treatment	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
Group 1 (control)	42.53±0.81	37.43±0.22	45.95±1.33	42.58±1.04	14.100±0.50	13.96±0.43	15.63±0.23	13.75±0.43
Group 2 (125 mg/kg)	36.13±0.99*	36.33±0.44	51.00±0.74*	43.68±0.64	12.150±0.37	13.70±0.19	15.75±0.48	14.00±0.15
Group 3 (250 mg/kg)	44.38±1.50	38.48±0.31	48.00±0.38	44.68±1.20	14.475±0.78	14.60±0.26	15.18±0.19	14.48±0.38
Group 4 (500 mg/kg)	41.78±1.33	37.98±0.55	43.38±1.18	44.35±1.09	14.325±0.18	14.15±0.49	14.60±0.55	14.35±0.34

Values are given as mean ± SEM for each group. * indicate significant difference at $p<0.05$, compared to Group A (Control). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test.

MSEPO: Methanol Stem Extract of *Portulaca oleracea*

Table 2: Effect of different doses of MSEPO on RBC and WBC level

Parameter	Duration							
	RBC ($X10^{12}$/L)				WBC ($X10^{9}$/L)			
Treatment	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
Group 1 (control)	7.20±0.14	7.28±0.17	8.10±0.29	7.59±0.69	6.28±0.86	7.50±0.25	10.10±0.90	7.70±0.18
Group 2 (125 mg/kg)	7.06±0.42	6.82±0.11	8.13±0.22	7.71±0.09	9.20±0.84*	9.35±1.22	9.10±1.28	9.15±0.60*
Group 3 (250 mg/kg)	7.02±0.49	7.00±0.24	8.48±0.26	7.41±0.04	5.78±0.19	11.20±2.09	6.08±1.03	7.10±0.08
Group 4 (500 mg/kg)	7.22±0.30	7.53±0.17	8.06±0.23	7.47±0.04	10.13±0.57*	7.44±0.26	8.33±0.84	9.23±0.23*

Values are given as mean ± SEM for each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval (p >0.05). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. RBC: Red Blood Cell; WBC: White Blood Cell; MSEPO: Methanol Stem Extract of *Portulaca oleracea*

Table 3: Effect of different doses of MSEPO on PLT and NEUT level

Parameter	Duration							
	PLT ($X10^{9}$/L)				NEUT ($X10^{9}$/L)			
Treatment	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
Group 1 (control)	783.25±12.98	658.25±12.13	780.75±25.64	724.75±6.34	1.55±0.21	3.88±2.87	2.13±0.34	2.18±0.08
Group 2 (125 mg/kg)	587.50±46.26*	730.50±20.55	776.30±228.20	764.50±11.90	3.00±0.18*	1.31±0.05	1.85±0.36	2.70±0.11*
Group 3 (250 mg/kg)	879.75±34.80	623.00±11.31	579.40±202.82	693.50±3.97	1.70±0.13	2.63±0.58	1.08±0.31	2.18±0.09
Group 4 (500 mg/kg)	933.50±15.13*	797.50±37.98*	952.50±34.56	669.00±16.64*	2.50±0.13*	1.96±0.28	1.45±0.17	3.30±0.12*

Values are given as mean ± SEM for each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval (p >0.05). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. MSEPO: Methanol Stem Extract of *Portulaca oleracea*; PLT: Platelet, NEUT: Neutrophil

Table 4: Effect of different doses of MSEPO on LYMP, MID and MCV level

Parameter	Duration											
	LYMP ($X10^{9}$/L)				MID($X10^{9}$/L)				MCV (fL)			
Treatment	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
Group 1 (control)	2.83±0.10	26.59±19.71	7.33±0.76	4.65±0.17	0.43±0.06	0.70±0.37	0.85±0.03	0.48±0.05	58.98±0.52	52.05±0.82	57.33±0.21	59.00±0.29
Group 2 (125	5.58±0.51*	10.11±0.55	7.85±0.35	6.30±0.21*	1.03±0.06*	0.39±0.21	0.98±0.09	0.73±0.06	59.88±0.97	55.88±0.62	196.65±135.78	55.98±0.47*

mg/kg)												
Group 3 (250 mg/kg)	3.68±0.19	12.11±0.97	4.93±0.45*	4.25±0.18	0.58±0.09	0.58±0.06	0.63±0.09	0.70±0.13	62.35±1.39	53.20±1.28	60.75±0.33	58.30±0.18
Group 4 (500 mg/kg)	6.88±0.33*	6.95±0.36	6.85±0.28	5.150.21	0.90±0.09*	0.30±0.05	0.63±0.06	1.03±0.05*	61.40±1.14	54.95±1.33	59.38±0.70	57.55±0.16*

Values are given as mean ± SEM for each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval (p >0.05). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. MSEPO: Methanol Stem Extract of *Portulaca oleracea*; LYMP: Lymphocytes; MID: Midsized cells (monocytes); MCV: Mean Corpuscular Volume

Table 5: Effect of different doses of MSEPO on MCH, MCHC and MPV level

Parameter	DURATION											
	MCH (pg)				MCHC (g/dL)				MPV (fL)			
Treatment	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
Group 1 (control)	18.93±0.35	18.63±0.20	18.83±0.15	18.50±0.30	32.25±0.12	35.41±0.30	32.05±0.41	32.85±0.34	8.25±0.64	6.55±0.14	8.75±0.20	8.30±0.09
Group 2 (125 mg/kg)	18.83±0.23	20.10±0.42	19.00±0.11	17.78±0.11	33.08±0.51	36.88±0.27*	31.88±0.82	33.40±0.75	8.30±0.11	6.23±0.09	8.45±0.16	8.53±-.11
Group 3 (250 mg/kg)	19.48±0.18	21.05±0.48*	19.23±0.25	19.23±0.17	32.60±0.22	36.83±0.38*	32.58±0.21	33.55±0.83	8.60±0.11	6.53±0.11	9.03±0.94	8.30±0.58
Group 4 (500 mg/kg)	19.28±0.23	19.60±0.45	18.25±0.37	17.70±0.57	33.38±0.39	37.75±0.30*	30.90±016	32.83±0.47	8.33±0.09	6.58±0.09	8.68±0.13	8.45±0.15

Values are given as mean ± SEM for each group. Experimental groups are compared with group A (control). No significant difference at a 95% confidence interval (p >0.05). P: statistical level of significance as determined by one-way Analysis of Variance (ANOVA) followed by Tukey's post-hoc test. MSEPO: Methanol Stem Extract of *Portulaca oleracea*; MCH: Mean Cell Hemoglobin; MCHC: Mean Cell Hemoglobin Concentration.

4. Discussion

The synthesis of several powerful and effective pharmaceuticals now in use originated from plants (Gurib-Fakim, 2006). Additionally, traditional medicine plays a significant role in meeting the healthcare requirements of poor nations (Hosseinzadeh *et al.,* 2015). Nevertheless, despite the significant financial gains associated with medicinal plants and their perceived safety and lack of toxicity, previous studies have demonstrated their involvement in the development of different types of toxicity (Obidike & Salawu, 2013), highlighting the urgent need to investigate their potential toxicity (Arome & Chinedu, 2013). The objective of this study is to evaluate the impact of the methanol stem extract of Portulaca oleracea on hematological parameters. Through a sub-chronic toxicity test, we want to determine the safety of this plant and make suggestions for its safe usage in medical applications. The objective of this research was to assess the impact of stem extracts from Portulaca oleracea on hematological markers. Blood is an essential instrument used to evaluate the

physiological and pathological condition of vertebrates (Obinna & Kagbo, 2018). The composition of blood varies depending on the health condition of the organism (Dkhill, 2011). This may be attributed to changes in the structure of cells, the capacity of membranes to allow substances to pass through, and the metabolic processes, as well as exposure to harmful chemicals (El-Aziz *et al.,* 2014). The research found that the stem extracts of Portulaca oleracea had a significant ($p<0.05$) effect on the percentage of haematocrit at 125mg/kg on day 15 and day 45. However, there was no significant difference in the mean levels of Haemoglobin and RBC between the test groups and the control group. There was a statistically significant ($p<0.05$) increase in the variance of the mean white blood cell (WBC) count on day 15 and day 60, for the doses of 125mg/kg and 500mg/kg body weight, respectively. A raised leucocyte count indicates not just illness or stress, but also a response to a medicinal treatment, particularly steroids, which stimulate greater production of white blood cells (Okoye *et al.,* 2016). The findings indicate that extracts from the stems of Portulaca oleracea may possess the ability to enhance the formation of white blood cells. This effect is likely due to the presence of phyto-steroidal compounds in the extracts. Soetan *et al* (2013) found that animals with a high white blood cell (WBC) count are able to produce antibodies by phagocytosis, resulting in a strong immune response.

The findings of this study, which involved the administration of MSEPO, align with the results reported by Oyedeji and Bolarinwa (2012). In their study, they observed no significant variation in the haematological parameters of rats given doses of 25, 50, and 75 mg/kg bw of methanol extract of aerial parts of *Portulaca oleracea* for 30 days. Nevertheless, the current investigation demonstrated that the extract may enhance the formation of white blood cells when the time of exposure is extended, as seen on day 15 and 60 at the respective doses of 125mg/kg and 500 mg/kg bw. In contrast to our results, Shafi and Tabassum (2015) observed a significant reduction in white blood cell count in mice when administered a 400 mg/kg bw dosage of a 50% ethanol extract of the whole plant for a duration of 14 days. The difference seen may be attributed to the plant sections used in the investigations, since the distribution of phytochemicals varies across various plant parts. The claim is substantiated by the research conducted by Ezeabara *et al* (2014), which showed varying quantities of phytochemicals and nutrients in different regions of Portulaca oleracea.

5. Conclusion

According to the findings of this research, it can be concluded that the stem extracts of Portulaca oleracea, as utilized in the experiment, did not have any harmful impact on the haematological parameters. Therefore, it can be said that these extracts are non-toxic to the blood of the experimental animals and, by extension, to people.

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