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Effects of methanolic curry leaf extracts (*Ocimum irvinei)* on the haematological parameters of Wistar rats

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Abstract

This study was set to evaluate the effect of administration of the methanolic extract of *Ocimum Irvinei* leaf on the haematological parameters of both male and female wistar rats. Previous reports have shown that this plant species is a good medicinal plant which has been used since antiquity for various ailments and also has a rich embodiment of nutritional and phytochemical constituents. Its application is mostly seen as a spice in most of dishes due to its flavouring. The haematological parameters analysed were Haemoglobin (HGB), Platelet Distribution Width (PDW), Platelet Large Cell Ratio (P-LCR), Procalcitonin Test (PCT), Haematocrit (HCT), Mean Platelet Volume (MPV), Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Red Blood Cell Count (RBC), Red Cell Distribution Width (RDW), and White Blood Cell Count (WBC) with Mindray BC-2800 Auto Haematology Analyser. Result obtained from the study showed that there was very little difference in the haematological parameters across groups and between both sexes. This infers that administration of the leaf extract of O. irvineihas little or no pronounced effect on the blood cells. Since there was very little change observed in the results of the test subjects, it is recommended that this study should be carried out for a longer period of time (a year or more) to stand the chance of getting more pronounced results. When compared with the Rat Reference Range shown in chapter one, there were several notable differences between the results and the normal range; while some where relatively higher or lower, some were well within the range.

Keywords: Wistar Rat; Hematological; *Ocimum Irvinei*; Methanolic

1. Introduction

Ocimum Irvinei is grown mainly in southern Asia and north-eastern Africa for its fragrant lemon scent, and it's used for cooking. It is used in small quantities as a natural flavouring ingredient dueto its distinctive aroma in food preparation and for preservation purposes. Several recent worldwide studies in the area of medicinal plants in different fields of medicine (plant-based medicines, health products,pharmaceuticals, food supplements and cosmetics) has identified that several fruits and vegetables serve as rich and natural sources of various bioactive and phytochemical compounds (Khaki and Fathiazad, 2010), which confers on them the ability to be used in the treatment of several human diseases (Sarojini and Manavalan, 2012). The bark and roots are used as a stimulant, and externally to cure eruptions and bites of poisonous animals; while the leaves have been reported to enhance digestive secretions and relieve nausea, indigestion, and vomiting (Khadar et al; 2012). Blood is a vital connective tissue, composed mostly of cells suspended in a fluid-like intercellular substance known as plasma. One of its primary functions includes maintaining homeostasis (Khadar, et al; 2012).

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Haemoglobin concentrations are quite valuable in monitoring feed toxicity, especially with feed constituents that affect the blood and overall health status of farm animals (Khadar et al; 2012). According to (Khadar et al; 2012) red blood cells are involved in the transportation of oxygen and carbon dioxide in the body. Thus, when the blood cell count is reduced, it implies that there's a reduction in the level of oxygen that would be carried to the tissues, as well as the carbon dioxide that's sent back to the lungs (Khadaret al; 2012). The main function of the white blood cells is to fight infections, defend the body by phagocytosis against foreign invasion, and to produce or at least transport/distribute antibodies in immune response. Animals with low WBC count are usually exposed to high risk of diseases and infections, while the ones with high counts have a higher degree of resistance to diseases. They also have enhanced adaptability to local environment, and early detection of foreign bodies. Based on reports made by Harley et al; 1992, it stated that PCV, Hb, and MCH are the major indices used for the evaluation of circulatory erythrocytes, and are essential in the diagnosis of anaemia. They also serve as useful indices for the capacity of bone marrow to produce red blood cells in mammals (Harley et al; 1992). The aim of this research is the evaluation of the effects of methanolic extract of *Ocimum irvinei* on the haematological parameters of albino wistar rats. However, interspecific hybridization and polyploidy, common occurrences within this genus (Harley et al; 1992) have created taxonomic confusion making it difficult to understand the genetic relationship between many types of basil (Grayer et al; 1996). According to the literature, some of the species of this genus showed significant variation in both morphology and essential oil components. The Phytochemical study of *Ocimum basilicum* and *O. sanctum* showed that they contained many terpenoids, eugenol, geraniol, linalool, and eucalyptol as their major constituents (Vasudevan et al., 1999). Other compounds such as camphor, limonene, α-pinene, β-selinene, and camphene were also found in O. irvinei (Verma et al., 2015). The pharmacological effects produced on the bacterial strains Staphylococcus aureus and Pseudomonas aeruginosa was determined when standard antibiotics and O. irvinei essential oils were combined. *Ocimum irvinei* essential oil associated with existing standard antibiotics may increase their antibacterial activity, resulting in a synergistic activity against bacterial strains of clinical importance. The antibacterial activity of O. irvinei essential oil may be associated with linalool (Araujo, et al.; 2016). The antimicrobial activities of chloroform, acetone and two different concentrations of methanol extracts of Ocimum basilicum L. were studied. The cells of microorganisms, which were treated and untreated with plant extracts, were observed by using the scanning electron microscope. It was observed that the treated cells were damaged (Kaya, et al.; 2008).

2. Materials and methods

2.1. Sample Collection

Freshly harvested leaves of Ocimum irvinei were bought on the 25th of May 2018, from Oyigbo Market. The identification was carried out at the Reference Herbarium for Research and Germplasm Conservation, Department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt.

2.1.1. Preparation of the Ocimum irvinei leaf extract

The fresh leaves bought from the market were sorted out and left to dry until zero water content was found in it. The dried leaves were then grounded into powdered form with the help of a blender (manual).

The fine powder was then soaked or dissolved in absolute methanol in an extraction vessel for about 72 hours during the period of maceration, and the extraction was done by the aid of a rotary evaporator. The extract was then dried in a water bath, after which it was diluted with DMSO (Dimethyl sulfoxide) before being administered to the test animals.

2.1.2. Collection of Experimental Animals

A total number of 40 rats (20 males and 20 females) weighing between 180-200g was used for this experimental research. The animals were procured from the Animal House of the Department of Physiology, in the Faculty of College of Health Science, University of Port Harcourt, and were used throughout the study.

2.1.3. Acclimatization of the Animals

The animals were kept according to their sexes in clean and dry wire mesh cages in an environment of normal room temperature, where they were exposed to 12 hours light/dark cycle under the standard conditions, and left to adapt to their new environment for a week (7 days). During this period, they had access to water and food (standard finisher diet, Top feed, Nigeria) ad libitum, throughout the duration of the study.

The male and female rats were kept in separate cages to prevent them from mating and reproducing, since it's not part of the study being carried out.

Due to limited amount of cages in the animal house, the rats were placed ten-ten in a cage (a total of four cages), and differentiated by colouring their skin.

2.2. Experimental Design

After the period of acclimatization, the animals were then divided into eight groups of male and female.

The extract administration was done by oral gavage; carried out once daily for 14 days (two weeks), while the dose of the extract used for this study was based on previous studies which used Methanol leave extract of *Ocimum* species in determination of several biological parameters in wistar rats.

According to Saha, Ghosh, and Nathan (2012), the 100 mg/kg body weight dose is the non-toxic range; hence this study employed ranges below the non-toxic level (50mg/kg) and above the non-toxic level (150mg/kg).

2.3. For the Male Rats

- **Group 1 (Control)** – consisted of 5 rats weighing between 180-200g, and received only feed and distilled water daily throughout the study.
- **Group 2** – consisted of 5 rats weighing between 180-200g, and received 0.06ml of 50mg/kg of the extract orally once a day (morning) with feed and water.
- **Group 3** – consisted of 5 rats weighing between 180-200g, and received 0.12ml of 100mg/kg of the extract orally once a day (morning) with feed and water.
- **Group 4** – consisted of 5 rats weighing between 180-200g, and received 0.18ml of 150mg/kg of the extract orally once a day (morning) with feed and water.
- **For the Female Rats**
- **Group 1 (Control)** – consisted of 5 rats weighing between 180-200g, and received only feed and distilled water daily throughout the study.
- **Group 2** – consisted of 5 rats weighing between 180-200g, and received 0.06ml of 50mg/kg of the extract orally once a day (morning) with feed and water.
- **Group 3** – consisted of 5 rats weighing between 180-200g, and received 0.12ml of 100mg/kg of the extract orally once a day (morning) with feed and water.
- **Group 4** – consisted of 5 rats weighing between 180-200g, and received 0.18ml of 150mg/kg of the extract orally once a day (morning) with feed and water.

2.4. Sample Collection and Determination

The administration of the Ocimum irvinei extract lasted a period of 2 weeks (14 days) immediately after acclimatization. And at the end of the experimental administration, the animals were anesthetized using chloroform and sacrificed humanely. The blood was drawn by cardiac puncture and emptied into the EDTA bottles. The blood samples were taken to the laboratory for haematological analyses with the aid of the automated haematological analyzer.

2.5. Determination of haematological parameters

The procedure for the haematology test was carried out using the Auto Haematology Analyzer.

2.5.1. Procedure

- Sample (blood) was collected and placed in an EDTA bottle and inverted to mix properly.
- The Haematology machine was switched on and allowed to complete the on process displaying the parameters on the screen and with the commencement of a prob (tube).
- The sample was introduced into the tube (prob) by placing the EDTA bottle containing the blood under the prob to make sure it touches the blood and the aspirator was pressed.
- The machine dispensed the sample into the various counting chamber compartments and each of the chamber aspirates the respecting three solution (E-Z cleanser, cell lyse and diluents).
- Each of the reagent mixes with the aspirated sample at the counting chamber for proper dilution of the aspirated blood samples.
- The counting was done by the machine automatically within it seconds and the machine ends counting process and display the result value.
- The value was printed by pressing the printing button and the printing rollers rolled out the result accordingly.
- The result was compared with the normal international value inbuilt in the machine.

3. Results and discussion

Table 1 Effect of the Administration of methanolic Ocimum *irvinei* Extracts on the White Blood Cells of Male Wistar Rats

Group (Male)	WBC	LYM%	MID%	NEUT%	LYM#	MID#	NEUT#
1	17.20±0.40	72.00±1.10	7.50±0.40	20.50±1.50	12.40±0.50	1.30±0.10	3.50±0.20
2	15.20±1.40	58.15±5.75[a]	6.70±0.20[b]	35.15±5.95 [c]	8.90±1.70	1.05±0.15	5.25±0.45
3	16.35±0.25	60.90±1.00	8.10±0.80	31.00±1.80	9.95±0.35	1.35±0.15	5.05±0.25
4	18.35±8.95	66.55±1.85	8.50±0.30	24.95±1.55	12.40±6.30	1.50±0.70	4.45±1.95

Table 2 Effect of the Administration of methanolic Ocimum *irvinei* Extracts on the White Blood Cells of Female Wistar Rats

Group (Female)	WBC	LYM%	MID%	NEUT%	LYM#	MID#	NEUT#
1	18.65±0.65	71.00±9.30	6.95±0.45	22.05±8.85	13.30±2.20	1.30±0.00	4.05±1.55
2	15.95±2.35	75.70±2.10	6.55±0.05	17.75±2.15	12.15±2.15	1.05±0.15	2.75±0.05
3	24.20±8.80	68.10±0.80	7.20±0.10	24.70±0.70	16.60±6.20	1.75±0.65	5.85±1.95
4	22.90±7.40	73.40±0.70	6.05±0.55	20.55±1.25	16.90±5.60	1.45±0.55	4.55±1.25

Table 3 Effect of the Administration of methanolic Ocimum *irvinei* extracts on the Red Blood Cells of Male Wistar Rats

Group (male)	RBC	HGB	HCT	MCV	MCH	MCHC	RDW-SD	RDW-CV
1	6.18±0.41	16.00±0.90	33.05±3.25	53.45±1.65	25.85±0.25	48.55±2.05	36.25±4.65	16.85±1.65
2	5.81±0.09	15.10±0.40	30.75±0.45	53.05±0.05	25.90±0.30	49.05±0.55	35.30±3.70	16.65±1.75
3	5.86±0.16	15.35±0.15	31.45±0.85	53.65±0.05	26.15±0.45	48.75±0.85	39.05±1.85	18.15±0.85
4	5.58±0.26	14.30±1.60	29.50±0.50	53.00±1.60	25.50±1.70	48.35±4.65	38.10±6.50	17.85±2.55

Table 4 Effect of the Administration of methanolic Ocimum *irvinei* extracts on the Red Blood Cells of female wistar rats

Group (femal)	RBC	HGB	HCT	MCV	MCH	MCHC	RDW-SD	RDW-CV
1	5.69±0.01	14.40±0.30	31.05±0.05	54.65±0.25	25.25±0.45	46.35±1.05	37.20±0.00	17.00±0.10
2	5.82±0.19	14.70±0.60	32.00±1.20	55.00±0.20	25.20±0.20	45.85±0.15	39.95±0.95	18.15±0.35
3	5.73±0.17	14.55±0.25	30.80±2.00	53.80±1.90	25.35±0.35	47.35±2.25	34.35±4.65	15.90±1.60
4	5.59±0.35	14.05±1.15	30.65±2.35	54.85±0.75	25.05±0.45	45.75±0.25	34.40±2.80	15.65±1.05

Table 5 Effect of the Administration of methanolic Ocimum *irvinei* extracts on the Platelets of Male Wistar Rats.

Group (Male)	PLT	MPV	PDW	PCT	P-LCR
1	291.00±4.00	8.70±0.20	9.20±0.00	0.25±0.01	20.25±2.85
2	399.50±60.50	8.55±0.15	9.85±0.35	0.34±0.06	18.10±1.90
3	409.00±57.00[d]	8.45±0.25	9.70±0.80	0.34±0.06	17.10±3.40
4	189.00±73.00	8.75±0.55	8.85±1.15	0.15±0.05	20.80±8.50

Table 6 Effect of the Administration of methanolic Ocimum *irvinei* extracts on the Platelets of Female Wistar Rats.

Group (Female)	PLT	MPV	PDW	PCT	P-LCR
1	325.00±19.00	8.45±0.15	10.10±0.90	0.27±0.02	17.70±2.40
2	396.00±68.00	8.85±0.45	10.75±0.55	0.35±0.08	21.05±4.75
3	440.00±29.00	8.55±0.35	10.50±1.30	0.37±0.04	18.25±3.55
4	332.00±37.00	8.55±0.05	10.35±0.15	0.28±0.03	19.40±0.50

The result obtained from this study as seen in Figure 1 shows that the White Blood Cells (WBCs) increased in a dose response manner in the male group, as it was seen to be higher in the high dose group and much lower in the low dose group. However, the Neutrophils decreased in a dose response manner where the low dose had the highest level and the high dose had a low concentration, close to that of the control. Also, the Basophils and Lymphocyte levels of Group 2 animals were seen to be significantly lower ($p<0.05$) when compared with the control (Table 1).

The WBCs of the female group administered with the extract on the other hand did not show any significant variation (Table 2) as they were all closely related to the control group and did not follow a particular trend However, in comparing the results from the male and female, it was observed that the lymphocyte level in the male animals treated with 50mg/kg BW and 150mg/kg BW of the extract was significantly lower ($p<0.05$) than with their female counterparts. The Neutrophil levels in group 2 male animals were also seen to be significantly higher 50mg/kg BW than the result obtained in the females.

Analysis of the result obtained for the red blood cell (RBC) parameters show that there was no significant variation in both male and female animals (Table 3 and 4). The results were also seen to be stable among the groups (Table 3 and 4). Also, in comparing the male and female result, there was no significant variation as both were of relative values.

The results of the analysis of the values of platelet parameters showed that the platelet level in group 3 animals were significantly higher ($p<0.05$) than those of group 4 which had the lowest value among the treated animals (table 5). The female group also showed similar trend but with no significant difference. However, in comparing the result obtained for both the males and females, it was observed that the group 4 males were significantly lower ($p<0.05$) than their female counterparts. The overall parameters remained relatively the same.

The lymphocyte percentage levels in both male and female groups were relatively higher than the other parameters. The lymphocyte numbers was also higher in the groups when compared with the Rat Haematological Reference range, with the exception of group 2 male, which was within the range. The increase in the lymphocytes could be as a result of bacteria and virus that triggered a response by the immune system that causes an increase in a certain type of White Blood Cell.

The neutrophil percentage in both male and female groups was also higher than the other parameters. The Mean Cell Volume (MCV), Mean Corpuscular Haemoglobin Concentration (MCHC), and Platelets also had high values than the others. Group 3 males and females had the highest platelet value when compared with the other groups.

When compared with the Rat Haematological Reference range, the Red Blood Cell (RBC) result levels were lower. The Haematocrit (PCV) was also lower, while the White Blood Cells (WBC) and Lymphocytes were higher than the reference range. The Haemoglobin (HGB) levels were within the normal reference range when also compared.

Group 1 male (M1) had a lower neutrophil percentage than the other test groups. Group 2 was higher than groups 1, 3, and 4. Group 3 was higher than group 4.

Group 4 male's White Blood Cell was higher than groups 1, 2, and 3. Also, the group 1males had a higher MID% than their counterparts in group 2, while group 4 had the highest. MID is Minimum Inhibitory Dilution in blood tests. They measure rare cells and precursor white cells. A high MID in blood tests means a high level of the rare cells. The MID cells may include less frequently occurring and rare cells correlating to monocytes, eosinophils, basophils, blasts and other precursor white cells. Some automated blood cell counting analysers can determine the relative size of the cells and are only able to identify neutrophils and lymphocytes. These analysers group everything that is not either a neutrophil or a lymphocyte into the MID category.

The normal range for Procalcitonin (PCT) is 0.10 – 0.28. The PCT levels of the group 1 and 4 male rats were within the range, while group 2 and 3 had higher levels. Group 1 and 4 females had normal levels, while group 2 and 3 had higher ones.

The normal range for Mean Platelet Volume (MPV) is 7.4 – 10.4. All the groups result fell within the normal range. The normal Platelet Distribution Width (PDW) range is 10.0 – 17.0, and all the groups had normal levels.

The normal Platelet range is 100 – 300. The female groups all had higher results than the reference range. The group 2 and 3 males also had higher platelet levels, while group 1 and 2 were within the normal range.

4. Conclusion

In conclusion, Haematological parameters were reported to be affected by many factors which include size, sex, age, physiological status, environmental conditions, and diet regimes like the quality and quantity of food dietary ingredient,

Findings from this study show that administration of the methanolic leaf extract of *Ocimum irvinei* had very little effect on the haematological parameters of both the male and female animals. Although there were some changes in some particular parameters, it wasn't enough to make much of a difference. From the table of results, the White Blood Cells were significantly higher than the normal reference range that was shown in the introduction.

When compared with the Rat Reference Range, there were several notable differences between the results and the normal range; while some where relatively higher or lower, some were well within the range. The Red Blood Cells were seen to be lower than the normal range too.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest were disclosed amongst authors.

Statement of ethical approval

Ethical approval was sought and received from Animal Ethics Committee and Departmental Head.

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