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Research Article

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Assessing the Impact of Aqueous Extract of *Pterocarpus mildbraedii on* the Haematological Indices of Male *Rattus novergicus* (Wistar Rats)

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Abstract The impact of the aqueous extract of Pterocarpus mildbraedii on the haematological profile and blood film of Rattus norvergicus (wistar rat) was investigated. A total number of 20 rats were acclimatized for two weeks and randomly distributed into four groups of five animals each from group A to D. The rats were administered 2ml of different protocol for sixty (60) consecutive days (once every 48hour). Group A was the control group, Group B, C and D were administered 0.04, 0.07 and 0.13g/mL of the leaf extract of P. mildbraedii respectively. After 60 days, the surviving rats were examined for haematological profiling and blood film. The result of the haematological analysis showed that the extract of P. mildbraedii administered to the wistar rats caused an increase in the white blood count 16.19±0.14× 109 cells/L and platelet count 900.95±23.05× 109 cells/L; while there was a reduction in the red blood count 7.10±0.60× 109 cells/L. The findings of the present study of the extract of P. mildbraedii on wistar rat reveal that the leaves alter the blood profile positively.

Keywords Pterocarpus mildbraedii, Rattus norvergicus, haematological, and Blood film.

Introduction

Plant based foods are recommended by nutritionist for human consumption to maintain healthy life. Plant based proteins and its derivatives are recommended as alternative to meat and dairy products. Eating plant-based food helps to reduce our individual carbon footprint, and it serves as mitigation measure to the changing climate (Gayatri et al., 2014: Newman et al., 2003).

Vegetables and mushrooms are major component of our diet and daily food, while fruits are eaten directly. Varieties of fruits are blended together as smoothes and it has become very common lately. Fresh juices are

extracted from fruits and it is widely replacing alcoholic beverages and soft bottle drinks. Herbs are dried and used as tea, some people bled it into powered form and add to meal, and most local people add it to alcoholic beverages. There is a general belief that these plants contain, antioxidants, mineral, nutrients, vitamins and other chemical substances that have the capacity to cure wide range of diseases and disorders. Most locals believe that these plants can boost immunity, remove free radicals that cause cancer, protect the nervous system, reduce inflammation, maintain the pH balance in the body, aid brain function, help the formation of collagen, help the heart to contract normally, prevent arthritis and help the joints to function properly (Gayatri et al., 2014: Newman et al., 2003). They also believe that these plants have capacity to regulate blood sugar, cure diabetics, help in blood yield, cure anaemia, and enhance sexual performance, increases libido and fertility. The native people eat this plant based on how easily they get them around their environment and their local knowledge of the plants (Jennifer et al., 2012). Some of these vegetables, fruits, mushrooms and herbs have been scientifically tested and proven to have chemical components and nutritional value that can help in fighting some diseases and profiled to be non-toxic, while many are yet to be proven scientifically.

A. Plant classification

P.mildbraedii belong to the family Fabaceae, sub family faboideae and it was recently assigned to the informal monophyletic Pterocarpus clade within the Dalbergieae. P. mildbraedii plant is very useful; the leaves are used as food and medicine. Extract of the leaves, barks and seeds are used for the treatment of many common ailments like headaches, pains, fever, anaemia, convulsions, and respiratory disorders.

1. Plant Features

According to Jennifer et al., (2012), P. mildbraedii is a fast growing plant with good coppice with superficial root system. The leaf flushes are intermittent patterned. The Flushes are green throughout the year and season when most vegetable had dried up. Oha plant still remains green and fresh. The tree is well above 30m tall with greyish or pale brown looking smooth bark, it exudes reddish gum when the bark is cut with a rounded crown. the Leaves alternate, imparipinnate, with a length of out 30-35 cm; the stipules lanceolate, is about 1 cm, caducous; leaflets is about (5–)7–16, alternate, is elliptically-oblong to ovate, about 6–15 cm × 3–8 cm, the base is rounded to cuneate, with abrupt acuminate apex. It poses a little-branched panicle of between 4–16 cm in length. The flowers are bisexual, papilionaceous, the merous is about 5; calyx is between 4–9 mm in length, the lobes inside are covered with thick short hair. It fruit is obovate-orbicular pod of between 9–13 cm in length, the wing with broad membrane with seed. (Jennifer et al., (2012).

A. Origin and geographical spread of the plant

Pterocarpus species are more than fifty-five (50), over twenty (20) of it are found in Africa. The two populations of P. mildbraedii in West-Central Africa and East Africa are sometimes considered as subspecies: mildbraedii and usambarensis. They differ in size of bracts and flowers. P. mildbraedii grows in Nigeria and many parts of Africa, it grows in the Republic Benin, Ghana, Sierra Leone, Liberia, Côte d'Ivoire and it has been spotted in central and eastern Africa countries like Cameroon, Tanzania along the Udzungwe and Usambaara Mountain, it also prospers in some region in Equatorial Guinea and Gabon (Akpanyung, et al, 1995).

B. Oha leaf (P. mildbraedii)

P. mildbraedii whose common name is Oha is a leafy green vegetable with nutritive and anti-nutritive potentials though is yet to be adequately scientifically investigated. The leaves are fresh and edible; they are used for soup preparation in the eastern part of Nigeria. It is a very delicious delicacy, and it is wildly acclaimed to have the potency to boost blood production and aid in the reproductive biology (system) of man (Dhellot et al., 2006.

C. Rationale of study

P. mildbraedii leaves are widely consumed by many in Southern Nigeria and other parts of West Africa and it is used and prescribed as herbal remedy by traditional medicine practitioners which are highly consulted by most Nigerians. Little is known about the potency of P. mildbraedii claims of boosting blood production and to curing anaemia.

D. Aim and objectives of study

The aim of this experiment is to access the impact of aqueous leave extract of Oha on the haematological indices of male wister rats. The present study is aimed at investigating the role of various doses of the extract of fresh P. mildbraedii leaves on the haematological and blood film of wistar rats.

E. The objectives are:

[1]. To determine the proximate and phytochemical composition of Oha leaf (P. mildbraedii)

[2]. To determine the effect of P. mildbraedii on the haematological indices of the wistar rats.

2. Material and Methods

A. Collection, identification, processing and extraction of plants

Fresh leaves of (P. mildbraedii) was collected from a farm settlement in Ogbulu village in Oshimili North Local Government Area of Delta state in July, 2021 and the identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria.

Plate 1a: Drying of plant

Plate 1b: pulverized to very fine particles

Plate 2: Weighting of fine particles of the leave

Plate 3

Plate 4: Acclimatization of the experimental rats **Plate 5**

B. Preparation of abatement

As shown in Plate 1a and 1b, the abatement was the leave extract of (P. mildbraedii). The leaves collected were air-dried to crispiness in the laboratory (preventing room temperature of 30 ± 2ºC for two weeks) as shown in Fig. 1a and in Figure 1b, the dried material were reduced to coarse form using a pestle and further pulverized to very fine particles using Viking Executive Jon cod pulverizing machine (Model: YLH2M2 – 4).Ten (10g) of the powder leaves was subjected to infusion extraction and exhaustively extracted with 0.5L of warm water for four hours as seen in Fig. 2. The extract was filtered and stored at temperature of about 4ºC in a clean container prior to use.

C. Collection and acclimatization of experimental rats

As shown in Plate 3, the entire experimental animals were conducted in accordance with standard guidelines (Council for International Organization of Medical Science (CIOMS), 2018 on use of animal for experimental toxicology study. Male wistar rats (6-7 weeks old) weighing within the range 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria. They were acclimatized for two weeks until they were 8-9 weeks and their weight taken. The animals were housed (Male only) in wooden cages with wire mesh covers. The

animals were fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo State, Nigeria) and given distilled water.

D. Experimental setup

The rats were distributed randomly into five (5) groups of five animals of group A TO D. The rats were administered different treatment protocol as stated below:

Group A – Control (CTR)
Group B – 0.04g/ml (OHA 1)
Group C – 0.07g/ml (OHA 2)
Group D – 0.13g/ml (OHA 3)

The rats were maintained in the laboratory condition; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo State, Nigeria). Each animal in a group was gavaged 2ml of the different protocol as described above for sixty (60) consecutive days (once every 48hour). At the end of the exposure period, survivors were fasted overnight and sacrificed under slight anaesthesia; then blood samples and organs were collected.

E. Haematological analysis

Haematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx Corporation Kobe, Japan) following the manufacturer's instructions. Briefly, the sample was mixed and placed in contact with the sample probe for aspiration, when the buzzer sounds twice 'beep, beep' and when the LCD screen display ANALYZING, the sample was removed. Following this, the units executed automatic analysis and the result was displayed on the LCD screen and printed out.

F. Data Analysis

All statistical analysis was conducted with statistical package for social scientist (SPSS) and Microsoft Excel Computer software. Data are represented as mean ± SE (n = 5/sex). One- way ANOVA was used to determine the differences among various groups.

3. Results and Discussion

A. Phytochemistry and Proximate Composition P. mildbreadii Leaves

Phytochemical screening of the aqueous leaf extract of P. mildbreadii, showed the presence of various secondary metabolites such as phenolics, alkaloids, tannins, saponins and flavonoids except for glycosides, steroids, triterpenes and phylobatnins which was absent in the leaf extract of P.3mildbreadii, (Table 1). The indicated that the leaves of P. mildbreadii, in percentage (%) was richer in Dry Matter (92.00 ± 1.00), Nitrogen Free Extract (39.06 ± 0.44), crude protein (25.94 ± 0.56) and dry matter (92.00 ± 1.00)

However, the percentage was lesser in Ash (7.00 ± 1.00) and Moisture (8.00 ± 1.00).

Table 1: Phytochemical composition of P. mildbreadii

	Parameter	*P. mildbreadii*
1.	Ash	6.3.00 ± 0.03
2.	Crude Fibre	17.61 ± 0.44
3.	Crude protein	25.41 ± 0.03
4.	Fat	9.66 ± 0.01
5.	Dry Matter	86.00 ± 1.00
6.	Moisture	8.00 ± 1.00
7.	Nitrogen Free Extract	39.06 ± 0.44
	Carbohydrate	19.6± 0.04

Table 2: Proximate Composition of P. mildbreadii

	Parameter	*P. mildbreadii*
1.	Flavonoid	+
2.	Alkaloid	+
3.	Tannins	+
4.	Saponins	+
5.	Phenolics	-

6.	Glycosides	-
7.	Steroids	-
8.	Triterpenes	-
9.	Phylobatnnin	-

NB: - indicates absent; + indicates present;

3. Blood Haematology

A. White blood cell count

Figure 4 depicts white blood cell counts of wistar rats administered aqueous extract of P. mildbreadii. The white blood cell of the rats rose to 15.65±2.25× 109 cells/L when it was compared with the Control 15.00±2.40× 109 cells/L. There was also significant increment in OHA 3 with recorded value of 16.19±0.14× 109 cells/L when 0.13g/ml was administered on the rats. Nevertheless, there was a decline in the white blood cell count of rats at OHA 1 value of (14.05±4.35× 109 cells/L) relative control 15.65±2.25× 109 cells/L.

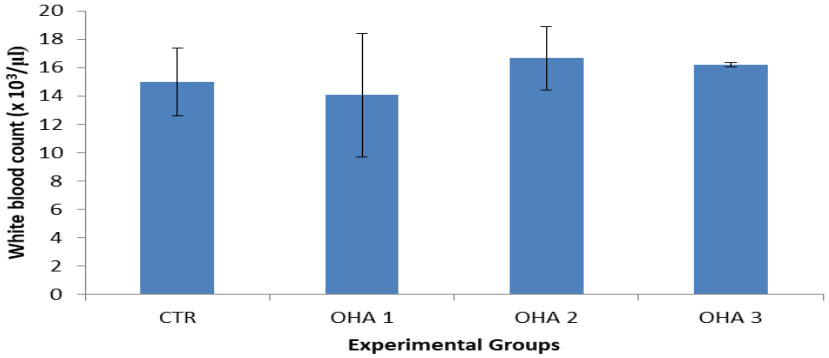


Figure 4: White blood cell count of wistar rats administered aqueous extract of P. mildbreadii

B. Lymphocyte count

Figure 5 depicts when, the lymphocyte count increased to 11.53±0.58× 109 cells/L when compared with the control C at a reading of 10.65±0.45× 109 cells/L. The lymphocyte count declined in OHA 1 to 7.60±1.30× 109 cells/L when 0.04g/ml was administered and in OHA 2 to 8.500±2.70× 109 cells/L. 0.07g/ml was administered.

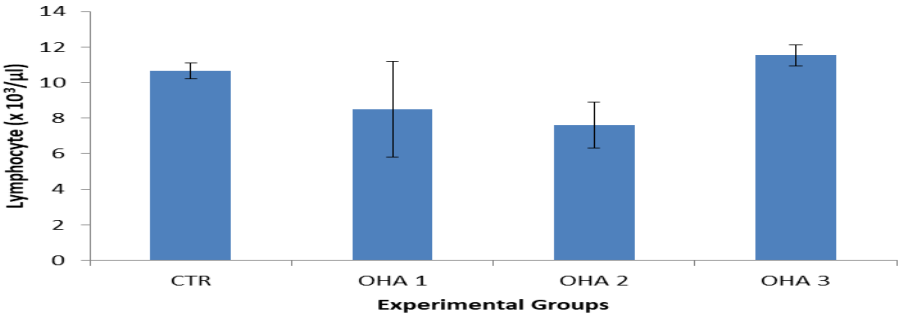


Figure 5: Lymphocyte count of wistar rats administered aqueous extract P. mildbreadii

C. Monocyte count

Figure 6 depicts increment in the monocyte count, when aqueous extract of P. mildbreadii, was administered to the rats in OHA 1, OHA 2 and OHA 3 when compared with the control C at a reading 2.65±1.15× 109 cells/L .OHA 3 has the highest value of 3.92±0.12× 109 cells/L when 0.13g/ml aqueous extract of P. mildbreadii was administered, followed by OHA 1 3.40±0.40× 109 cells/L when 0.04g/ml administered and OHA 2 was the lowest with monocyte count of 3.00±1.60× 109 cells/L with 0.07g/ml.

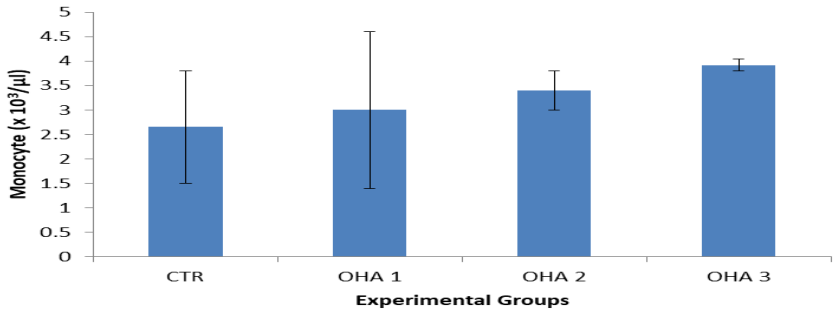


Figure 6: Monocyte count of wistar rats administered aqueous extract of P. mildbreadii

D. Granulocyte count

Figure 7 depicts granulocyte count result and it was noticed that aqueous extract of P. mildbreadii, administered caused progressive increase in granulocyte counts in. OHA 3 with value of 9.2±0.54× 109 cells/L when 0.13g/ml was administered on the rats while OHA 2 value 5.7±0.05× 109 cells/L when 0.07g/ml was administered and OHA 1 has a value of 2.65±0.55× 109 cells/L when 0.04g/ml of the extract was administered. This was all compared with the control C with value 1.70±0.80× 109 cells/L.

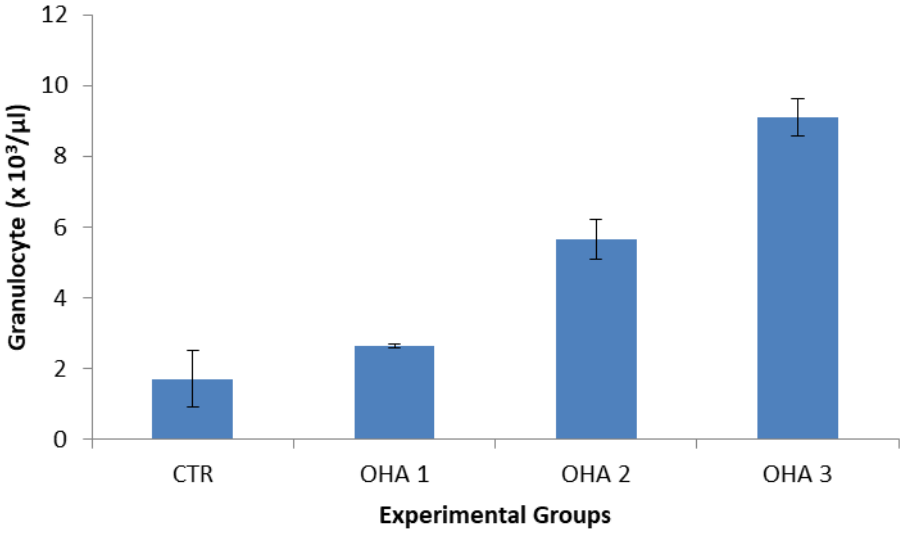
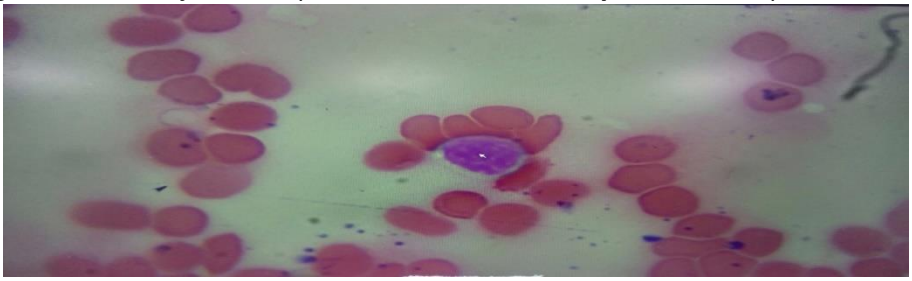
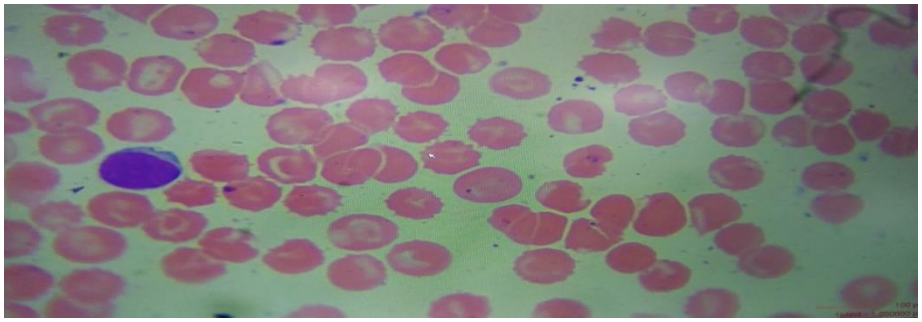


Figure 7: Granulocyte count of wistar rats administered aqueous extract of P. mildbreadii

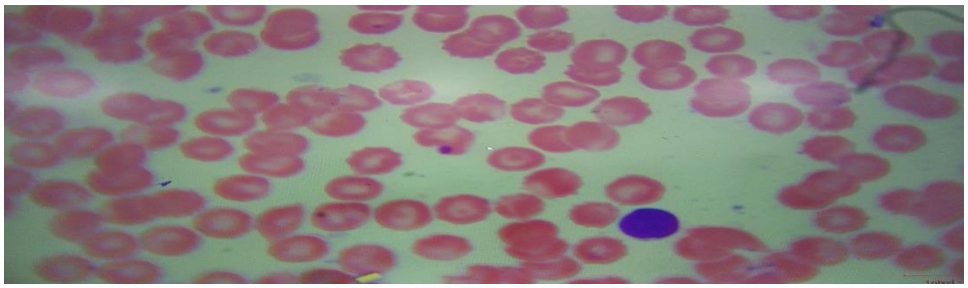


[1]. Plate 2: Haematological micrographs of control rats

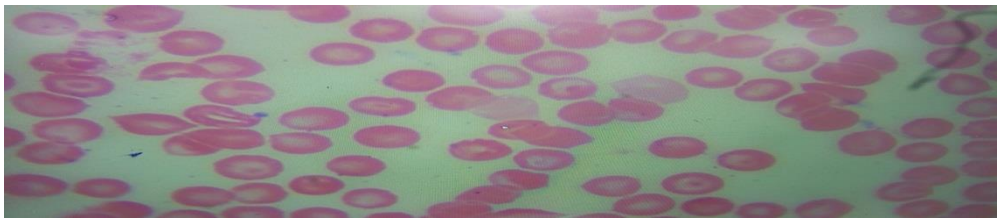
WBC showed Relative lymophocytosis about 81% and granulocytes 19%, no atypical cell seen, Eryhrocytes showed normocytic cells +++, normochromic cells+++, polychromatic cells +, Plateletes appeared adequate in number and evenly distributed



[2]. Plate 3: Haematological micrographs of rats administer 0.04g/mg of aqueous extract of Oha leaves (P. mildbraedii). WBC showed mild lymphocytosis normal in size 67%, normal sized neutrophils 33% Erythrocytes showed normocytic cells +++, normochromic cell +++, polychromatic cell++, target cells +, crenated cell +



[3]. Plate 4: Haematological micrographs of rats administer 0.04g/mg of aqueous extract of Oha leaves (P. mildbraedii). 7WBC appeared adequate in number with moderate increase lymphocytes but normal in size (69%), neutrophil 20% and 11%.no atypical cell seen, Erythrocyte showed normocytic cells +++, normochromic cell +++, polychromatic cell +, crenated ++, target +, Platelet appeared adequate in number and normal in size



[4]. Plate 5: Haematological micrographs of rats administer 0.13g/mg of aqueous extract of Oha leaves (P. mildbraedii). WBC appeared moderately increased but adequate in differential classifications, lymphocytes 50%, neutrophil 50%, Erythrocytes showed normocytic cell +++, normochromic cells +++, polychromatic cells ++, target cell +, Platelet appeared adequate in number and normal in size

4. Discussion

In this study the aqueous extract of P. mildbraedii induced Lymphocytosis, Granulocytosis, Monocytosis. This was also seen in the study undertaken by (Goh et al., 2016) in that study extract of P. mildbraedii, significantly increased white blood cell count when administered on rats.

The ability of the leaves extract of P. mildbraedii to boost white blood cell is as result of the presences of iron, and white blood boosting minerals it contains. This is helps protect the body against infections (Akpanyung et al., 1995). Again high concentration of lymphocyte in the blood could be as a result infection after trauma leading to high antibody production (Abbas and Lichtman, 2003). Increased granulocyte levels suggest a high cellular damage/inflammation and depressed immunity; while increased monocyte count might be a sign of a chronic infection, an autoimmune disorder or a blood disorder (Shugaba et al., 2012). Granulocytes such as neutrophils are the first-responders to inflammation and cell damage, while eosinophils are primarily associated with parasitic infections and an increase in their number may indicate presence of microbial in the body (Albert, 2005).

Ingestion of aqueous extract of P. mildbraedii may accelerate the metabolic rate, which increases in the generation of free radicals and cell demage. The immune system auto responds and neutrophils quickly congregate at a focus of infection, attracted by cytokines expressed by activated endothelium, mast cells and macrophages (Ear and McDonald, 2008). Monocytes help the immune system to fight infections. The white blood cells have the ability to turn into antigen presenting cells and macrophages when the immune system detects a foreign substance. These cells identify antibodies and communicate with B and T cells build immunity to combat the foreign substance in the body. Macrophages are specialized cells involved in the detection, phagocytosis and destruction of bacteria and other harmful organisms (Ugochukwu, 2003).

The reduction in hemoglobin concentration and red blood cell counts suggest blood loss and body inability to store iron this could be caused by ulcers trauma, some form of cancer and in women loss of blood during the menstruation period disrupted hematopoietic process. The presence of heavy metals alters the blood biochemical pathway and a blood disorder Porphyrias caused by the deficiency of enzyme or substrate also attributes as well (Aminat et al. 2021,)

The reduction of hemoglobin, red blood cell and hematocrit could be caused by bleeding, malnutrition or as result of kidney disease (cirrhosis), cancer and medication used in treating cancer. Iron and vitamins deficiency such as folate, vitamin B12 and vitamin B6 and not enough nutrients in food we eat. Similar findings have also been reported by (Hounkpatin et al., 2013) suggesting toxicity of cadmium, mercury and their combination as a factor for the reduction of hemoglobin, red blood cell and hematocrit the study revealed a significant decrease in red blood cells (RBC), haemoglobin concentration (HGB) in wistar rats.

5. Conclusion

The results of this study indicated that the aqueous extract of P.mildbraedii is a good food supplement for prevention and treatment of anaemia. This study revealed that P. mildbraedii leaves is also a good source of protein, amino acid, vitamins and minerals and it should be consumed for healthy living because of his haematopoietic properties. No lethality was observed in rats administered extract P.mildbraedii. Animals did not show any significant changes in behavioural, physiological and physical activities.

6. Recommendations

[1]. Bioactive products from P. mildbraedii leaves are safe and non-toxic. Oha leaves are a highly nutritious and medicinal and it is recommendation for consumption for healthy life.

[2]. P.mildbraedii leaves are available in the market collected from the wild but they are not easily found in home gardens like other green leafy vegetable. A better understanding of the variation of the species will help in the process of domestication.

[3]. Farmers are advised to cultivate this plant as researchers are focused on validating it quality, medicinal and therapeutic potency for the development of herbal drugs which are fast replacing the conventional drugs.

[4]. The development of alternative medicine is highly supported by the World Health Organization (WHO). The World Health Organization on its own surveys estimates that about 80% of the global populations rely mostly on traditional medicine for their primary health care needs. And in the nearest future most of the world population will depend on plant-based medicine (WHO, 2001).

References

[1]. Albert B.S., (2005). Pollution potential of leachate from selected solid waste dumpsites in Kakuri Kaduna, Nigeria. Journal of Engineering and Applied Sciences 3:45-51

[2]. Aminat E., Agbozu I.E. and Nwosisi M. (2021). Determination of Pollution Index between Active and Closed Dumpsites in Port Harcourt Metropolis. Journal of Chemical, Biological and Physical Sciences 5(2): 2051-2061.

[3]. Akpayung I.O, Udoh A.P, and Akpan E.J., (1995). Chemical composition of the edible leaves of Pterocarpus mildbraedii, Plant Foods Human Nutrition (Netherlands) International Journal of Pharmaceutics 48:2O9-215.

[4]. Dhellot G., Shyamala, R and Manikandan, R (2006) Determination of bioactive compounds in Ziziphus oenoplia leaves extract using gas chromatography and mass spectroscopic technique: Journal of Pharmacognosy and Phytochemistry 2019; 8(5): 157-160

[5]. Ear M.J., and McDonald T., (2008). Brine shrimp toxicity evaluation of some Tanzanian plants used raditionally for the treatment of fungal infections. Afr. J. Tradit. Complement. Altern. Med. 4:219-225.

[6]. Gayatri N, Mruntyanjay S, Rahani KS (2014). Antioxidant Potential and Nutritional Values of Vegetables: A Review. Res. J. Med Plants 8:50-81.

[7]. Hounkpatin N.C, Salunke V.R, Bhinse SB, and Yadav A.V., (2013). Cardiotonic activity of aqueous extract of heartwood of Pterocarpus marsupium. Indian J. Exper. Biol. 45(6):532-537.

[8]. Jennifer SJ, Elizabeth DN, Elvin A (2012). Factors related to fruit, vegetable and traditional food consumption which may affect health among Alaska Native People in Western Alaska. Int. J. Circumpolar Health 71:17345. Lichtman, A.O., 2003.

[9]. Nutritional potentials of some tropical vegetable leaf meals: Chemical characterization and functional properties. Afr. J. Biotechnol., 5: 49-53.

[10]. Newman, O.O.A., B.L. Fetuga and V.A. Oyenuga, (2003). Accumulation of mineral elements in five tropical leafy vegetables as influenced by nitrogen fertilization and age. Scient. Hortic., 18: 313-322.

[11]. Shugadaba H.I., Ifon E.T, and Bassir O., (2012). The nutritive value of some Nigerian leafy vegetables- part 2: The distribution of proteins, Carbohydrates (including ethanol-soluble simple sugars), Crude fat, Fibre and Ash. Food Chem. 5: 231-235.

[12]. Ugochukwu K.J., (2003). Nutritional value of Balsam Apple (Momordica balsamina L.) leaves. Pak. J. Nutr. 5(6): 522-529.

[13]. World Health Organization (2001) Geneva Legal Status of Traditional Medicine and Complementary/Alternative Medicine: A Worldwide Review. World Health Organisation, Geneva, pp: 129-143