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Effect of Hydroethanolic Extract of *Fleurya Aestuans* on Haematological Parameters and Oxidative Indices of Phenylhydrazin Induced Toxicity

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Fleurya aestuans popularly called West Indian Wood Nettle is known for its therapeutic potential as it has been long used by herbalists in managing many diseases. The haematinic efficacy of the leaves of this plant was examined in this study. A total of 43 rats were procured, 13 rats were used for lethal dose (LD_{50}) studies while the remaining 30 rats were grouped into six groups of five rats each and allowed to acclimatize for two weeks. Groups 1 served as control and were administered 2ml/kg of the extract vehicle, group 2 served as negative control, groups 3 and 4 served as test group and were administered with 50 mg per body weight of *Fleurya aestuans* leaf extract and 200 mg per body weight of same extract, while group 5 and 6 were administered with 0.23 ml per body weight of bioferon and 100 mg per body weight of Kaempferol respectively. Phenyl hydrazine was used to induce the anaemia in all the groups; the extract was prepared and administered orally using oral gavage. Blood samples were collected through ocular puncture after 14 days and analysed for haematological parameters using standard manual methods. Results showed that the extract significant ($P < 0.05$) enhanced the low levels RBC counts, Hb concentration, PCV, SOD,

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CAT and GSH levels but reduced the abnormally elevated levels of MCV, MCHC, MDA, WBC and Platelets counts. The oral administration of *Fleurya aestuans* leaf extract could be useful both in boosting blood parameters and combating diseases irrespective of the anaemia and leukocytosis caused by phenylhydrazine therapy.

Keywords: Fleurya aestuans; Hydro-ethanolic; Haematological parameters; Oxidative indices.

1. INTRODUCTION

The use of herbs for the treatment of human diseases is as old as humanity. Throughout the years, scientific data has reported on some of the commonly utilized therapeutic plants. It has been estimated that about two third of the global populace especially in the developing countries rely on herbs as their main source of health care (citation). Therefore, there is need for more investigation on various therapeutic plants in the management and amelioration of diseases, particularly the chronic ones [1].

Fleurya aestuans is a therapeutic plant typically utilized by traditional medicine practitioners to treat and counteract ailments in West Africa; yet it has apparently gotten little scientific attention. This plant known as West Indian wood nettle is a somewhat yearly blossoming plant that normally grows 3 meter tall with stem that is fleshly and green in color [2]. *Fleurya aestuans* is innate to tropical Africa, however can be found in the Western half of the globe, eastern side of the equator and subtropics. In West Africa, the plant has been utilized as an abortifacient, antimicrobial, purgative, diuretic to treat blenorrhoea and chest issues, pneumonic and stomach inconveniences, looseness of the bowels and diarrhea [3]. There are additionally reports that leave concentrate of *F. aestuans* is utilized to treat joint pain, iron deficiency, hay fever, kidney issues, pains and inflammation [4]. The gastro-defensive properties of the methanol leaf concentrate of *Fleurya aestuans* in rodents has been described [5].

Phytochemical screening of the leaf of this plant by gas chromatographic flame ionization detector (GC-FID) has revealed the presence of alkaloids, saponin, flavonoids, cardiac glycosides, steroids and terpenes [6]. Likewise, atomic absorption spectrophotometer, hints at Chromium (Cr), Manganese (Mn), Nickel (Ni), Copper (Cu), Cadmium (Album), Iron (Fe), Zinc (Zn) and Lead (Pb), Potassium (K), Sodium (Na), Calcium (Ca) and Magnesium (Mg) in the plant's leaf [7] Studies on the antioxidant potentials of the leaf of this plant using 1,1-diphenyl-2-picryl hydroxyl quenching (DPPH) assay, superoxide scavenging assay, scavenging capacity towards hydroxyl ion (OH) radical, nitric oxide (NO) radical inhibition, hydrogen peroxide inhibition activity, lipid peroxidation (LPO) inhibition activity and 2,2'-azinobis-3 ethylbenzothiozolin-6-sulfonic acid (ABTS) cation decolourization test showed that the extract exhibited high scavenging activity against 1,1-diphenyl-2-picryl hydroxyl (DPPH), superoxide (O_2), hydroxyl ion (OH^-), nitric oxide (NO), hydrogen peroxide (H2O2), 2,2'-azinobis-3-ethylbenzothiozolin-6-sulfonic acid (ABTS) and Lipid peroxide (LPO) respectively [8]. Other studies have reported its antibacterial and antifungal potential. Different studies have revealed its antibacterial and antifungal potential [9,10]. However, studies on the efficacy of the leaves of this plant on some haematological parameters have been scanty. The present study examines the possible effect of the aqueous leaf extract of the plant on haematological parameters of phenylhydrazin induced toxicity in experimental animals.

2. MATERIALS AND METHODS

2.1 Preparation of *Fleurya aestuans* Leaf Extract

Fleurya aestuans leaves were obtained from University of Port-Harcourt, Nigeria and its environs and authenticated by a plant taxonomist, of the Department of Plant Science and Biotechnology, University of Port Harcourt. The plant was given herbarium number of: UPH/P/263.

The mature leaves were washed in tap water to remove debris and dried at room temperature over a period of three (3) weeks. The dried mature leaves were processed to fine powder utilizing manual granulating machine. 350g of the plant powder was measured and the weighed quantity of the plant powder was dissolved in 400ml of Water-Ethanol mixture (25:75) for 72 hours in an Extraction Jar. During this period it was well macerated to enable it absorb the solvent. After which it was sieved utilizing a Whatman No 1 filter paper to obtain the filtrate from the residue.

After obtaining a clear filtrate, it was emptied into an evaporating dish which was then dried on a steam bath at a temperature of 45^{o}C. The drying was monitored until it turned into a paste form. The yield of the crude ethanolic extract of *F. aestuans* leaves obtained weighed 75.3g and was preserved in a household refrigerator at 4^{o}C.

2.2 Lethal Dose (LD_{50}) of the Extract

The oral lethal doses (LD_{50}) of FAE in the rats were determined using the method described by Lorke [11] with slight modifications.

This method employed two phases;

Phase 1

In this phase nine (9) male rats were used. They were grouped into three (3) groups of three (3) rats each. Each group of animals received 40, 400 and 600 mg/kg of hydro-ethanolic extract of the leaves of *Fleurya aestuans* respectively. The animals are placed under observation for 24 hours to monitor their behavior as well as mortality rates.

Phase 2

In this phase four (4) male rats were distributed into four (4) groups of one (1) rat each. The rats were treated with 800, 1300, 3500 and 4600 mg/kg of the leaves of hydro-ethanolic extract of *Fleurya aestuans* respectively and then observed for a day for any sign of acute intoxication as well as mortality.

The LD_{50} of the extract was calculated using the formula below:

$LD_{50} = \sqrt{(D_0 x\ D_{100})}$

Where;

D_0 = Highest dose that gave no mortality,
D_{100} = Lowest dose that produced mortality.

2.3 Experimental Design

A total of 30 Wistar rats weighing between 120.7 - 200.5g obtained from the animal house, Madonna University Elele, Nigeria were used for the study. The rats were housed in wire meshed cage under standard conditions (temperature 25-29°C and natural dark/light cycle and fed with a standard rat pelleted diet and tap water ad libitum. The animals were given a period of two week for acclimatization.

2.4 Animal Placement/ Inducement

After acclimatization, the rats were weighed and randomly assigned into six groups (n=5 in each group). Using the method of Pooja *et al. [12]* anaemia was induced in all test groups (Groups 2-6) by intraperitoneal injection of phenylhydrazine at 40 mg/kg body weight on day 0 and additional doses were given on day 1 at 9am and 6pm respectively. The rat groups were treated as follows:

Group 1 (Control group) were only allowed rat feed and tap water ad libitum for 14 days.

Group 2 (PHZ only group) were only treated with PHZ.

Group 3 (Low dose extract group) were administered 50mg/body weight of *Fleurya aestuans* leaf extract for 14 days.

Group 4 (High dose extract group) were administered 200mg/body weight of *Fleurya aestuans* leaf extract for 14 days.

Group 5 (Positive Control group 1) were administered 0.23mg/body weight of Bioferon for 14 days.

Group 6 (Positive Control group 2) were administered 100mg/body weight of Kaempferol for 14 days.

2.5 Sample collection/ Analysis

24 hours after the last administration of the extract or drug, all experimental animals were anaesthetized using chloroform. 4 ml of blood sample was collected by ocular puncture from each of the animals using capillary tube and transferred into EDTA sample bottles and were analyzed for haematological parameters using a haematology analyzer (Mindray Auto Hematology Analyzer, BC-5200, USA) following the manufacturer's instructions. The parameters analyzed include white blood cell count (WBC) and the differentials, platelets, red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). Antioxidant status was analyzed using diagnostic kits from Randox laboratory UK.

3. RESULTS

All Results obtained from the study were presented in Tables and expressed as mean ± standard error of mean (M±S.E.M) as below.

The outcome demonstrated that the oral LD_{50} of *Fleurya aestuans* extract (FAE) did not produce any signs of toxicity in rats even up to the dose of 4600 mg/kg. Table 1 shows a statistically significant decrease ($p<0.05$) in MCH and MCHC at the lowest dose extract group (30.00 ± 06.41, 38.29 ±0.11) in contrast to PHZ only group (38.03±03.50, 46.29 ±0.02). At the highest dose extract group, there was a significant increase ($p<0.05$) in RBC counts, Hb, PCV (7.00 ± 0.22, 48.50 ± 1.24, 16.02 ± 0.10) and a remarkable decline in MCV, MCH and MCHC (77.20 ± 3.09, 28.08 ± 30.13, 37.20 ±0.05) in contrast to the PHZ only group (4.80 ± 0.09, 39.00 ± 0.58, 13.0 ± 0.19, 88.12 ± 2.53, 38.03±03.50, 46.29 ±0.02). MCV and MCHC decreased significantly ($p<0.05$) in the positive control group 1 in contrast to group 2. However, PCV increased significantly while MCV and MCHC decreased remarkable in group 6 when compared to group 2.

Table 2 shows a significant decrease ($p<0.05$) in Monocytes (10.20±0.05) and increase in Platelets (220.33±1.00) at low dose extract group in contrast to PHZ only group (7.54 ± 0.00, 265.20±1.05). At high dose extract group, a significant decrease ($p<0.05$) in WBC, Lymphocytes, Platelets and increase in Neutrophils were observed in contrasts to PHZ only group. At the Positive control group 1, Neutrophils, Eosinophil and Monocytes increased significantly ($p<0.05$) while Platelets decreased in contrast to PHZ only group. At the Positive control group 2, WBC and Platelets decreased significantly ($p<0.05$) in contrast to PHZ only group.

Table 3 shows that GSH significantly increased ($p<0.05$) at the low dose extract group in contrast to PHZ only group. CAT, SOD and GSH increased significantly while MDA decreased remarkably at high dose extract group in contrast to the PHZ only group. In the Positive control group 1, GSH increased significantly in contrast to PHZ only group, however, MDA decreased significantly while CAT, SOD and GSH increased significantly in the Positive control group 2 in contrast to PHZ only group.

3.1 Histology of the Spleen

Fig. 1. displays the photomicrographs of transverse sections of the rat's spleen in the control group and test groups.

4. DISCUSSION

The present study was aimed to examine the effect of hydro-ethanol effect of *Fleurya aestuans* leaves in phenylhydrazine induced haematological poisoning in Wistar rats.

Phenylhydrazine is a substance metabolite utilized broadly in the drug, agrochemical and chemical enterprises. Human presentation to PHZ happens basically by means of inhalation in the work environment, ingestion and skin contact. This agent is notable for its capacity to produce haemolysis in people and in rodents [13,14]. People's introduction to PHZ display bone marrow depression, as confirmed by anaemia (diminished RBC counts), leukocytosis (expanded WBC counts), as well as thrombocytosis (expanded platelet counts). A concealment of every one of the three components is called pancytopenia.

In the reports of Kolawole *et al.* [15] and Shukla *et al.* [16] treatment with PHZ exhibited factually huge reduction in erythrocyte parameters, for example, Hb levels, RBC counts and PCV levels which is in accordance with the current finding.

A decrease in erythrocyte parameters is an indicator of anaemia though the unusually raised levels of RBC indices proposes a disequilibrum in body water, megaloblastic anaemia and blood sepsis, whereas, the factually high expansion in WBC counts and platelets counts is an indication of a viral, fungal, bacterial or parasitic infection and iron deficiency and hemolytic anaemia respectively.

Reactive oxygen species (ROS), like superoxide anions, are under typical physiological conditions cleared by anti-free radical cells, for example, GSH, SOD and CAT. Superoxide anion is dismutated to hydrogen peroxide (H_2O_2) in a cycle catalyzed by SOD, and H_2O_2 is then cleared by catalase or GSH [17].

The values of SOD and GSH-Px were brought down in PHZ treated rats showing a decreased cell reinforcement limit. If not cleared by cellular anti free radical agents, superoxide anions can produce the exceptionally reactive and poisonous hydroxyl radical (−OH) through the Haber–Weiss response, which is catalyzed by iron [18].

Table 1. Values of hydro-ethanolic leaf extract of *Fleurya aestuans* on Phenylhydrazine induced erythrocyte toxicity

GROUPS	RBC ($x10^{12}$/l)	PCV (%)	Hb (g/dl)	MCV (fl)	MCH (pg)	MCHC (g/dl)
Group 1 (Control group)	6.20 ± 0.20	46.05 ± 1.30	15.30 ± 0.11	83.80 ± 2.04	32.32 ± 13.00	38.28 ±0.02
Group 2 (PHZ only group)	4.80 ± 0.09[a]	39.00 ± 0.58[a]	13.0 ± 0.19[a]	88.12 ± 2.53	38.03±03.50	46.29 ±0.02[a]
Group 3 (Low dose extract group)	6.10± 0.40	44.00 ± 1.31	14.70 ± 0.12	86.04± 6.13	30.00 ± 06.41[b]	38.29 ±0.11[b]
Group 4 (High dose extract group)	7.00 ± 0.22[b]	48.50 ± 1.24[b]	16.02 ± 0.10[b]	77.20 ± 3.09[b]	28.08 ± 30.13[b]	37.20 ±0.05[b]
Group 5 (Positive control group 1)	5.80 ± 0.50	43.00 ± 1.24	14.30 ± 0.23	81.10 ± 4.00[b]	33.04 ± 17.03	33.20 ±0.22[b]
Group 6 (Positive control group 2)	6.50 ± 0.17	45.40 ± 1.04[b]	15.70 ± 0.55	70.22 ± 3.19[b]	37.11 ± 37.02	38.20 ±0.00[b]

KEY:Values are presented as mean ± sem. n= 5. [a] *= mean values are statistically significant compared to control,* [b] *= mean values are statistically significant compared to PHZ treated groups*

Table 2. Values of hydro-ethanolic leave extract of *Fleurya aestuans* on Phenylhydrazine induced leukocyte toxicity

GROUPS	WBC ($x 10^9$)	Neutrophil (%)	Eosinophil (%)	Lymphocyte (% ±)	Monocytes (%)	Platelets ($x 10^9$)
Group 1 (Control group)	7.50 ± 1.00	35.00± 2.09	2.01 ± 0.12	58.31 ± 1.04	5.80 ± 0.09	216.00± 8.02
Group 2 (PHZ only group)	13.20±0.08[a]	28.00±3.38[a]	3.03± 0.50	62.22±1.02	7.54 ± 0.00	265.20±1.05[a]
Group 3 (Low dose extract group)	8.20 ± 0.04	25.00 ±2.15	5.97 ± 0.22	60.00 ± 2.02	10.20±0.05[b]	220.33±1.00[b]
Group 4 (High dose extract group)	6.00±4.80[b]	38.00±1.99[b]	4.04±0.00	50.50±0.15[b]	8.10± 0.26	198.00±5.11[b]
Group 5 (Positive control group 1)	9.80±4.80	35.00±1.99[b]	5.60±0.08[b]	50.10±2.25[b]	10.95±0.04[b]	218.22±0.61[b]
Group 6 (Positive control group 2)	7.20±0.50[b]	30.00 ± 1.24	2.00±0.23	63.00±4.00	5.00±17.03	235.20±0.22[b]

KEY:Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to PHZ treated groups

Table 3. Values of hydro-ethanolic leaf extract of *Fleurya aestuans* on some antioxidants levels of Phenylhydrazine induced hematotoxicity

GROUPS	MDA (nmol/mgprotein)	CAT (U/ml)	SOD (U/ml)	GSH (mg%)
Group 1 (Control group)	4.02 ± 0.30	30.80 ± 0.02	13.30 ± 1.04	38.54 ± 4.07
Group 2 (PHZ only group)	11.33±2.00[a]	18.23±0.30[a]	5.55±0.44[a]	20.66±0.50[a]
Group 3 (Low dose extract group)	5.10±0.00	24.06±0.90	10.30±0.20	33.30±0.10[b]
Group 4 (High dose extract group)	3.00±0.05[b]	27.19±0.11[b]	13.06±0.70[b]	41.80±2.00[b]
Group 5 (Positive control group 1)	8.11±4.80	20.00±1.99	9.60±0.08	28.10±2.25[b]
Group 6 (Positive control group 2)	1.22 ± 0.50[b]	31.03 ± 0.00[b]	13.88 ± 0.17[b]	37.22 ± 1.40[b]

KEY:Values are presented as mean ± sem. n= 5. [a] = mean values are statistically significant compared to control, [b] = mean values are statistically significant compared to PHZ treated groups

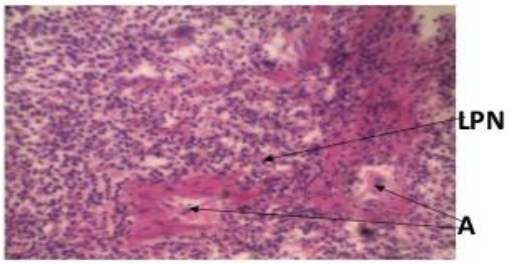

Fig. 1a.

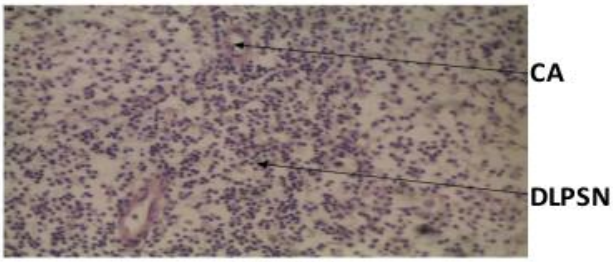

Fig. 1b.

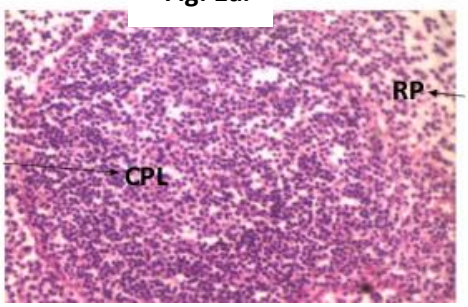

Fig. 1c.

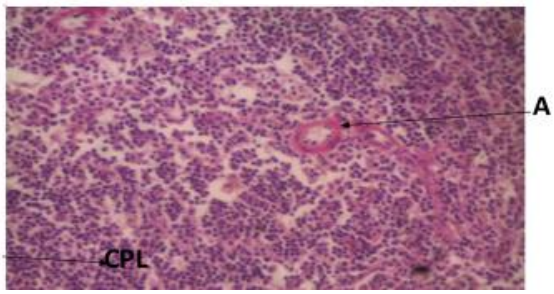

Fig. 1d.

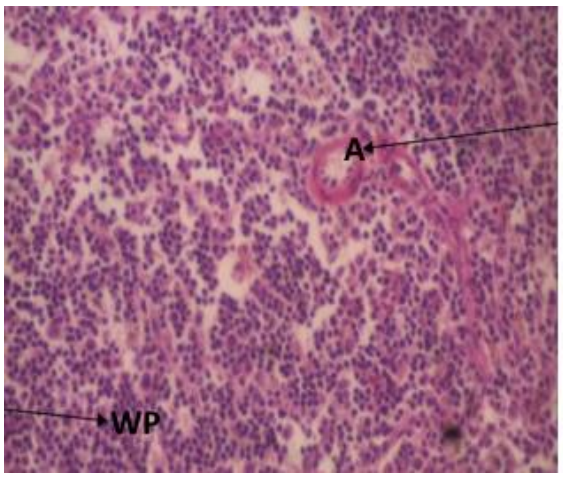

Fig. 1e.

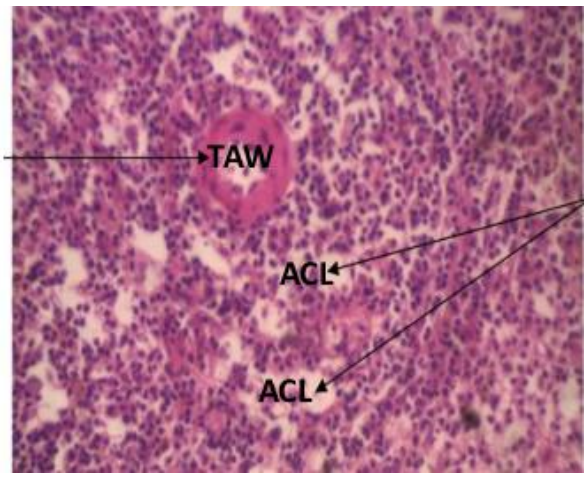

Fig. 1f.

Fig. 1. Photomicrographs of transverse section of the spleen of rats in 1a (Control) – shows that the white pulp consists of closely packed lymphocytes with prominent nuclei and defined cell outline; lymphocytes surrounds an arteriole forming the Malphigian corpuscles, while the red pulp consists of venous sinusoids separated by cords of Bilroths. 1b (PHZ only group) - shows poorly stained white pulp characterized by dispersed lymphocytes and lost cells. The venous sinusoids of the red pulp are collapsed; the cords of Bilroths are sequestered; Malphigian corpuscles show areas of cell loss. 1c (Low dose extract group) - shows white and red pulp which is similar to the control group. 1d (High dose extract group) - shows normal white and red pulp, White pulp show closely packed lymphocytes, an arteriole is seen at the center of Malphigian corpuscle. 1e (Positive control group 1) – white pulp shows thick walled arteriole that is dilated, lymphocytes are prominent, 1f (Positive control group 2) - white pulp shows normal Malphigian corpuscles with a centric arteriole. Lymphocytes are closely packed as in the control group

Key: LPN= Lymp with prominent nuclei, CA= Collapsed arteriole, A= Arteriole, DLPSN= Dispersed lymp with poorly stained nuclei, CPL= Closely packed limp in WP;
WP= White pulp, ACL= Area of cell loss, A= Arteriole, TAW= Thickened arteriolar wall

Expanded ROS levels inside the cell lead to oxidation of macromolecules, including lipids, nucleic acids, and proteins, there by upsetting cell capacities. MDA is a habitually utilized marker of lipid peroxidation and as indicated by reports, MDA levels were raised in separated rodent organs after PHZ treatment [19].

These discoveries showed that some level of oxidative stress and cell harm occurred in the rodent organs during PHZ treatment. The alleged harm to the blood vessel endothelium by PHZ might be because of the generation of free radicals, bringing about lipid peroxidation. In this examination, we noticed and exhibited that treatment with concentrate of *Fleurya aestuans* leaves standardizes the values of hematological and anti-oxidative stress markers.

There were outstanding reclamation of the altered blood and anti-free radical agents to values like that of the group 1 rats following *Fleurya aestuans* extract therapy and this efficacy by the extract might be because of the presence of the generally archived phytochemicals set up by the authors of the current examination.

Flavonoids, catechin, tannins and other phenolic compounds are notable regular cancer prevention agents. These constituents might be liable for its anti-anaemic as well as anti-leukemic action against phenylhydrazine. Antioxidant activities of the leaves of *Fleurya aestuans* extract against oxygen free radicals has been established by certain researchers including Okereke and Elekw [8] Ganiyat *et al.,* [9] which is consistent with the current examination.

In particular, the natural flavonol-3,4,5,7-tetrahydroxyflavone (kaempferol), the catechins, resveratrol, lycopene and various phenolic compounds, for example, p–coumaric, vanillic acid and tannic acid were portrayed in the leaves of *Fleurya aestuans* [6] and ensnared in their antioxidant actions and membrane protection [20] which is the situation of the current examination.

Further, the leaves of *Fleurya aestuans* extract may have stimulated erythropoiesis or potentially invigorated the secretion of erythropoietin in the kidneys. This accordingly, recommends a stimulatory impact of the extract on the bone marrows and kidneys respectively.

Erythropoietin provocation and discharge causes expedient generation of RBC [21] Tannins likewise present in the extract bind to proteins and sugars which are constituents of the erythrocyte layer and in this way may avert breakdown of the erythrocyte membrane. Accordingly, flavonoids and tannins which have been depicted to have concrete free radical clearance could inhibit peroxidation of polyunsaturated fats in the cell layer of RBC and subsequently, ward off haemolysis of RBCs [22]. Alkaloids are known to inhibit cyclic adenosine monophosphate (cAMP) phosphodiesterase thereby causing an accumulation of cAMP levels. This stimulates protein phosphorylation and synthesis with a possible enhancement of erythropoiesis [23].

These findings were in concurrence with the discoveries of Mohammad *et al. [13]* and Anslem *et al.* [24] in which plant extract turned around the impacts of phenylhydrazine induced haematotoxicity in laboratory rats.

5. CONCLUSION

This examination accordingly shows that ingestion of *Fleurya aestuans* notwithstanding the dose could be valuable both in fighting infections and in boosting blood parameters in iron deficient patients, thus, approves its utilization in botanical medicine and herbalism.

FUTURE PROSPECTS

The study therefore suggests the use of plant in correction of Anaemia. The dose in human being trial can be useful for the patients who are having iron intolerance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The research was approved by our institutional ethical committee. International standard, rules

and guidelines for use of animal for research was adhered to as approved by the committee [25].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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