AS MEN OF HONOUR WE JOIN HANDS
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Original Research

HAEMATOLOGICAL AND *IN-VIVO* ANTIOXIDANT MODULATORY ACTIVITIES OF *JUSTICIA SECUNDA* VAHL [ACANTHACEAE] LEAF EXTRACT IN PHENYLHYDRAZINE-INDUCED ANEMIC RATS.

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ABSTRACT

Background: Medicinal plants represent an important resource in the fight against diseases and, search for new lead compounds in the drug industry. Research into ethnomedicinal claims made about these plants serves as a key starting point in the investigation of their phytotherapeutic potential. The leaf of *Justicia secunda* is used ethnomedicinally in many climes to treat anaemia. This study was aimed at evaluating the anti-anaemic and *In-vivo* antioxidant activities of the methanol leaf extract of *J. secunda* (MEJS) in phenylhydrazine-induced anaemic rats.

Methods: Anaemia was induced in Wistar albino rats by intraperitoneal administration of 60 mg/kg of Phenylhydrazine (PHZ) for two consecutive days. This was followed by treatment with 100, 200 and 400 mg/kg doses of MEJS and 300 mg/kg ferrous sulfate, which served as the standard drug for two weeks. Blood samples were collected on days 0, 7 and 14 post-induction of anaemia and analyzed for changes in haematological parameters and antioxidant activities.

Results: Administration of PHZ led to a significant ($p < 0.05$) decrease in the number of red blood cell counts, haemoglobin and hematocrit concentration and an elevation of the mean cell volume. PHZ administration equally led to a significant reduction in the activities of Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) enzymes. The extract showed a significant reversal of the anaemic effects induced by PHZ as well as elevated the *In- vivo* antioxidant activities of SOD, CAT, and GPx. It equally caused a reduction in levels of lipid peroxidation as evidenced by decreased levels of malondialdehyde.

Conclusion: The methanol extract of *J. secunda* leaf has the potential to be useful in the management of acute anaemia, at a level comparable to that of ferrous sulfate, used in the management of iron deficiency anaemia.

Keywords: *Justicia secunda*, Phenylhydrazine, Anaemia, Anti-anemic activity, Oxidative stress, Antioxidants

INTRODUCTION

Anaemia, a public health problem that affects populations is defined as a decrease in the total number of circulating red blood cells (RBC), the concentration of haemoglobin (Hb), and/or percentage of packed red cells in a sample of centrifuged blood[1,2]. It is characterized by a decrease in the ability of the blood to carry oxygen due to a deficiency in the quality and quantity of haemoglobin, a molecule found in red blood cells[3]. Clinically, it manifests as fatigue, lethargy, depression, impaired cognitive function, insomnia, dizziness and in severe cases palpitation and loss of consciousness[3]. It is often overlooked, unrecognized and undertreated, particularly when it co-exists with more serious and chronic health conditions such as cancer, chronic kidney disease, congestive heart failure, rheumatoid arthritis, vasculitis and inflammatory bowel syndrome[4]. Major causes of anaemia include excessive blood loss, decreased and faulty production of red blood cells and, increased destruction of red blood cells. Risk factors for anaemia include poor nutrition, viral, bacterial and parasitic infections, pregnancy and presence of other chronic disease conditions such as chronic kidney disease, cancer, liver disease, inflammatory bowel syndrome, hepatitis C and auto-immune deficiency syndrome (AIDS)[3]. According to the World Health Organization (WHO), over one-third of the world population, amounting to over 2 billion people suffers from anaemia, with pre-age school children, pregnant and non-pregnant women representing the most vulnerable groups[1]. The incidence and burden of anaemia are higher in developing countries, a situation made worse by an inadequate supply of essential nutrients from diets or other sources and, high prevalence of gastrointestinal infections due to parasites which often results in heavy blood loss as well as conditions such as malaria and Haemoglobinopathies[2,5]. Clinical consequences of anaemia in pregnant women include increased chances of abortion, preterm delivery and birthing of babies with low weight[6]. It increases the need for blood transfusion before surgery, and predisposes to increased morbidity, mortality and length of hospital stay perioperative, as well as contribute significantly to associated tiredness and health-related poor quality of life following surgery[4]. Anaemia is also associated with considerable economic burden and loss as a result of the cost of acquisition of drugs, blood, transfusion and hospitalization, as well as decreased productivity, loss of work.

A direct correlation between hemolytic and some other forms of anaemia and oxidative stress has been established[7]. Inability on the part of a biological system to detoxify reactive intermediates and/or repair damages that results from the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in situ can lead to conditions that manifest as oxidative stress[8]. An antioxidant is a molecule strong enough to donate a molecule to a rampaging free radical and neutralize it, thus reducing its capacity to damage cells. The body counteracts oxidative stress by producing antioxidants, which are either naturally generated in situ, or externally supplied through foods and/or supplements. The major enzymatic antioxidants directly involved in the neutralization of ROS and RNS in the body are superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRx)[9]. The level of lipid peroxidation as measured by the concentration of malondialdehyde (MDA) is also used as a measure of *in-vivo*-antioxidant activity.

Medicinal plants have been used by most cultures as a source of medicine from the earliest times and, have become an important part of primary health care[10]. They are used to manage various kinds of disease conditions including, diabetes, hypertension, pain, inflammation and anaemia. *Justicia secunda* is a representative of plants used in ethnomedicine to manage anaemia.

Justicia secunda, known commonly as "Hospital too far", "Blood leaf" or "Blood tonic" has varied folkloric uses which include analgesia and inflammation, cough, cold, fever, malaria, measles, whooping cough, diarrhoea, hypertension, treatment of afterbirth problems, dysmenorrhea, and for dilation and curettage after miscarriage. A leaf decoction of the plant is used in the management of anaemia[11]. The anti-sickling, haematinic, anti-inflammatory, antinociceptive, *In*

vitro antioxidant, antimicrobial, antihypertensive, hypoglycaemic and anti-hyperglycemic activities of *J. secunda* have been reported[12-16]. Luteolin, aurantamide acetate, auranamide, quindoline, pyrrolidone derivatives, secundarellone A, B and C are compounds that have been isolated from the plant[17]. A previous study has documented the haematinic properties of the leaf of the plant in normal rats[13]; however, there is no report of the activity of the plant in any form/type of induced anaemia and in-vivo antioxidant activity. This study was therefore designed and carried out to evaluate the effects of the methanol extract of *J. secunda* leaf (MEJS) on phenylhydrazine (PHZ) induced anaemia as evidenced on the haematological parameters and, i*n vivo* antioxidant activities in rats.

MATERIALS AND METHODS

Methods

Plant collection and authentication

The plant was collected in Port Harcourt, Rivers State, Nigeria in February 2018 and authenticated by Dr A.O Osiyemi of the Plant Herbarium Unit, Forest Research Institute of Nigeria [FRIN], Ibadan Oyo State, Nigeria where a voucher specimen was deposited, and the herbarium number FHI112104 was issued.

Plant preparation and extraction

Leaves were separated from the stalk, washed under running water and dried under shade for three weeks, and further in an oven at 40°C for 30 mins before milling. Dried plant material was ground to a fine powder and stored in airtight glass bottles at 4^{0}C till needed. The powdered plant material (632 g) was extracted with methanol (5 L) in a Soxhlet apparatus for 12 hrs, and the extract obtained was concentrated *in vacuo,* (yield 16.12%) and stored in a glass bottle at -4°C till it was needed for use.

Experimental Animals

Ten weeks old male and female Wistar albino rats (180 – 220 g) were obtained from the Department of Pharmacology animal house and housed in same in plastic cages, with wood shavings as beddings. They were allowed to acclimatize for two weeks before use. Animals were maintained under an environmentally controlled temperature of 25 ± 5^{0}C, a relative humidity of 75 ± 5% and natural day and night light cycles of approximately 12/12 hrs. Water and animal chow were readily accessible to the animals' *ad libitum*. Ethical approval with reference number EC/FP/018/06 was obtained from the Ethical Committee of the Faculty of Pharmacy, University of Benin and as much as possible, animals were handled as per international rules guiding the use of animals[18].

Experimental Procedure

The protocol, according to Johnson and Besselsen (2002),[19] was employed in this study. Briefly, animals were randomly divided into six groups of 18 rats each and treated as shown in Table 1;

Table 1: Grouping and treatment of experimental animals

S/N	GROUP	TREATMENT
1	Normal control	No treatment; No induction
2	Negative control	PHZ + distilled water (10 mL/kg)
3	Extract treated	PHZ + Extract (100 mg/kg)
4	Extract treated	PHZ + Extract (200 mg/kg)
5	Extract treated	PHZ + Extract (400 mg/kg)
6	Positive control	PHZ + Ferrous sulphate (300 mg/kg)

Anaemia was induced by intraperitoneal administration of 60 mg/kg of PHZ for two consecutive days following an overnight fast. All other administrations were via the oral route for 14 days and day 0 represented the first day following the 48 hrs of anaemia induction. Blood samples were collected on days 0, 7 and 14 post-induction.

Specimen Collection

At the end of each treatment period, six rats from each group were anaesthetized in a chloroform chamber, and blood was collected via the inferior vena cava. Blood samples for haematological analysis were collected into Ethylene diamine tetraacetic acid (EDTA) tubes while that for evaluation of antioxidant activity was collected in lithium heparin tubes. The heparinized blood was centrifuged at 3000 rpm (LC-04R-N Centrifuge; Zenith Laboratory, China) and resulting plasma was used for the analysis of antioxidant activity.

Haematological Analysis

Blood samples collected were analyzed for the following Haematological parameters using an automated blood analyzer (Sysmex XE-5000; Kobe, Japan); Red blood cell count, (RBC), Haemoglobin concentration (HGB) Hematocrit (HCT), White Blood Cell count (WBC), Platelet count (PLT), Mean cell volume (MCV), Mean Cell Haemoglobin (MCH), Mean cell Haemoglobin concentration (MCHC), Red cell volume distribution width (RDW) and Mean platelet volume (MPV).

Determination of Antioxidant Activity

Superoxide dismutase antioxidant activity was determined by means of commercially available assay kits (Ransod®; Randox Laboratory, UK) by following the manufacturer's instructions. Briefly, xanthine and xanthine oxidase were used to generate superoxide radical that reacted with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to give rise to the formation of a red dye (formazan). The absorbance of formazan was measured spectrophotometrically (Uv- visible spectrophotometer; PharmaSpec1700 Shimadzu, Japan) at 505 nm and the activity of SOD was expressed in U/mL.

The method of Koroliuk et al.,[20] was adopted to determine the CAT antioxidant activity. In summary, the sample was incubated with H_2O_2 (100 µmol/mL) and Tris-HCL buffer (0.05 mmol/L) for 10 mins. The reaction was terminated by the addition of ammonium molybdate, followed by the measurement of absorbance of the yellow molybdate complex formed at 410 nm. Catalase activity was defined as the amount of enzyme required to decompose1 µmol of H_2O_2 per min.

The activity of GP_x was determined by the use of a commercial assay kit (Ransel®-Randox Laboratory, UK). Sample (10μL) was mixed with 500μL of reagent R1 and 20μL cumene R2. The absorbance was measured at 340nm, and the GP_x activity was calculated according to the manufacturer's instruction. Enzyme activity was expressed as U/mL.

The level of MDA in the samples was determined by the method of Varsney and kale (1990)[21] This was carried out by measuring the level of thiobarbituric acid reactive substances (TBARS) generated, as an indication of lipid peroxidation. Quantification of the TBARS was determined at 532nm by comparing the absorption to the standard curve of MDA equivalents generated by acid-catalyzed hydrolysis of 1,1,3,3-tetra methoxy propane. To measure MDA level, a working solution containing 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25N hydrochloric acid was prepared. Sample (250μL) was mixed with the working solution (500μL) and placed in boiling water for 10min. After cooling, the sample was centrifuged at 3000rpm for 10min, and 200μL of the supernatant was transferred to microplates, and the optical density of samples was measured at 535nm. The values of MDA were expressed as μmol/L.

Statistical Analysis

Data are expressed as Mean ± Standard Error of Mean (SEM). Statistical analysis was carried out with One-way analysis of variance (ANOVA) followed by Dunnett's comparisons test, performed using Statistical Package for Social Sciences (SPSS) for windows version 17 (IBM, New York, USA).* $p \leq 0.05$ and** $p \leq 0.001$ were considered significant.

RESULTS

Effect of extract on Haematological parameters

The rats developed acute hemolytic

anaemia characterized by a significant decrease ($p < 0.05$) in RBC count, HGB concentration and HCT percentage on day 0 post-injection of PHZ compared to the normal control. By day 14 of treatment, there was a non-dose dependent increase in RBC count in the extract-treated groups and the positive control group. HGB was restored within two weeks in the negative control and extract-treated groups, which was significant compared to the PHZ toxic effect observed on day 0. The level of HGB observed after 14 days of treatment in animals that received 400 mg/kg of the extract was higher than that seen in the normal control, and ferrous sulphate treated animals. The percentage of HCT produced by 100 mg/kg dose of the extract on day seven was observed to be higher than that produced by 200 and 400 mg/kg dose of MEJS as well as the standard (Table 2a).

Table 2a: Effect of varying concentrations of MEJS on Red blood cells, Haemoglobin concentration and Haematocrit in PHZ-induced anaemic rats

Group/Dose/Day	Day 0	Day 7	Day 14
Red Blood Cells (x 10^6/µL)			
Normal Control	8.01±0.56	7.27±0.26*	8.17±1.31
Negative Control	3.09±0.20**	4.74±0.21*	5.30±.089
100 mg/kg	3.51±0.55*	5.18±1.17*	6.96±0.52
200 mg/kg	3.87±0.22*	4.49±0.09*	6.91±0.80
400 mg/kg	4.01±1.37*	4.93±0.21*	7.13±0.18
Ferrous sulfate (300 mg/kg)	3.84±0.50**	4.46±0.23*	6.73±1.40
Haemoglobin Concentration (g/dL)			
Normal Control	13.30±0.40**	12.83±0.55	14.30±1.22
Negative Control	3.83±0.17*	8.97±0.19*	13.20±0.83
100 mg/kg	5.50±0.46*	13.13±1.00	13.90±0.93
200 mg/kg	5.40±0.62*	11.13±1.10	13.00±3.87
400mg/kg	6.70±1.99*	11.97±1.20	16.00±1.19
Ferrous sulfate (300 mg/kg)	4.90±0.75*	11.70±1.10	13.67±1.07
Haematocrit (%)			
Normal Control	40.93±3.11	41.20±2.56	45.03±3.03
Negative Control	12.23±2.04**	38.90±1.61	47.87±3.92
100 mg/kg	15.43±2.44*	42.97±9.54	52.03±3.82
200 mg/kg	17.90±6.23*	37.33±0.20	46.50±9.94
400 mg/kg	20.27±0.80*	38.93±1.53	51.93±3.78
Ferrous sulfate (300 mg/kg)	14.67±1.03*	39.17±3.42	53.93±5.99

Key: Values are expressed as Mean ± Standard Error of Mean (SEM), n= 6, * = $p < 0.05$, ** = $p < 0.001$ (statistically significant).

An increase in WBC in the extract-treated, negative and, positive control groups was observed on day 0. This was restored to normal within the first week in the extract-treated and positive control groups, and there was no significant difference ($p < 0.05$) in WBC by day 14 in these groups compared to the normal control. Significant increases ($p < 0.05$) in MCV, MCH and platelet count were observed in the extract-treated, negative and positive control groups. The MCV and MCH reverted to normal in all the groups by day 14 except for the MCV in the negative control (Table 2b).

Table 2b: Effect of varying concentrations of MESJ on White Blood Cells, Mean Cell Volume, Mean Cell Haemoglobin and Platelet Count in PHZ – induced anaemic rats

Group/Treatment/Day	Day 0	Day 7	Day 14
White Blood Cell (x10			
Normal Control	12.73±0.82	12.13±0.82	10.47±2.14
Negative Control	19.40±2.20	9.37±1.39	11.77±2.44
100 mg/kg	47.17±5.82*	9.27±2,57	10.13±2.26
200 mg/kg	51.57±3.70*	8.17±1.71	7.87±2.62
400 mg/kg	44.47±7.90*	8.67±0.62**	6.70±0.95**
Ferrous sulfate	47.87±8.49*	7.13±0.64**	13.13±2.86*
Mean Cell Volume (fL)			
Normal Control	57.33±0.55	53.10±3.57	50.60±1.85
Negative Control	81.00±1.04*	72.86±0.40*	71.97±6.07*
100 mg/kg	83.13±0.43*	73.10±5.57*	47.50±1.32
200 mg/kg	83.70±1.62*	74.10±1.20*	43.97±1.51
400 mg/kg	77.40±2.01*	74.93±1.63*	44.63±0.67*
Ferrous sulphate (300 mg/kg)	85.60±1.00*	50.77±1.36*	43.17±1.26*
Mean Cell Haemoglobin (pg)			
Normal Control	33.40±0.74	33.23±0.91	31.70±0.55
Negative control	40.33±1.26	27.57±2.24*	27.70±1.90*
100 mg/kg	37.43±1.44	30.17±1.13	26.87±1.79
200 mg/kg	35.70±3.12	29.57±1.02	28.33±1.94
400 mg/kg	39.23±2.95	30.70±0.17	30.70±0.57
Positive Control	40.20±0.90	29.80±0.26	24.17±3.07
Platelet Count (x104cells/µL)			
Normal Control	495.30±32.77	493.10±20.56	449.00±23.48
Negative Control	1303.00±64.67**	1049.00±14.72**	654.30±40.80
100 mg/kg	515.30±32.04	1074.00±60.33**	613.30±9.94
200 mg/kg	483.30±21.04	1046.00±75.44**	602.00±67.18
400 mg/kg	545.30±34.49	1044.00±44.92**	931.70±15.72*
Ferrous sulphate (300 mg/kg)	253.30± 8.45	886.00±32.88*	652.00±18.06

Key: Values are expressed as Mean ± Standard Error of Mean (SEM); n = 6; *= p<0.05; ** = p<0.001 (statistically significant).

Table 2c: Effect of varying concentrations of MEJS on Mean cell Haemoglobin, Red blood cell distribution width and Mean Platelet volume

Group/Treatment/Day	Day 0	Day 7	Day 14
Mean cell heamoglobin Concentration (g/dL)			
Normal Control	33.40±0.74	33.23±0.91	32.70±0.55
Negative Control	40.33±1.26	27.57±2.24*	27.70±1.90
100 mg/kg	37.43±1.44	30.17±1.13	26.87±1.79
200 mg/kg	35.70±3.12	29.57±1.02	28.33±1.94
400 mg/kg	39.23±2.95	30.70±0.17	30.70±0.57
Ferrous sulphate (300 mg/kg)	40.20±0.90	29.80±0.26	24.17±3.07
Red blood cell distribution width (%)			
Normal Control	17.90±1.48	16.70±1.36	16.83±1.78
Negative Control	14.80±0.25	24.33±1.37	19.70±2.89
100 mg/kg	17.97±0.58	25.57±2.24	25.37±1.50
200 mg/kg	17.43±0.75	25.67±2.22*	22.33±2.09
400 mg/kg	17.60±0.76	31.07±1.92*	19.30±0.47
Ferrous sulphate (300 mg/kg)	16.73±0.09	27.90±0.85*	24.47±3.14
Mean platelet volume (fL)			
Normal Control	6.63±0.38	7.37±0.38	7.30±0.26
Negative Control	7.57±0.43	8.60±0.12	8.57±0.29
100 mg/kg	6.53±0.15	8.33±0.68	8.03±0.67
200 mg/kg	6.13±0.26	9.00±0.38	8.17±0.86
400mg/kg	8.60±2.10	17.57±0.24*	21.53±0.76*
Ferrous sulphate (300 mg/kg)	8.37±1.86	19.57±0.54*	18.10±1.08*

Key: results are expressed as mean ± Standard Error of Mean (SEM); n = 6; * = p <0.05, ** = p <0.001(statistically significant)

PHZ induction led to a significant reduction ($p < 0.05$; $p < 0.001$) in the plasma levels of SOD, CAT and GPx in all the PHZ-treated groups compared to the normal control on day 0.

Treatment with standard hematinic preparation and different doses of MESJ led to significant increases in activities of SOD, CAT and GPx as seen in increased levels of these enzymes compared to the untreated PHZ-induced rats on day 7.

At the end of the second week of treatment, however, there was no significant difference in antioxidant enzyme activity in the treated PHZ-induced rats, compared to the normal control animals except for the untreated PHZ-induced rats (Figure 1 – 3).

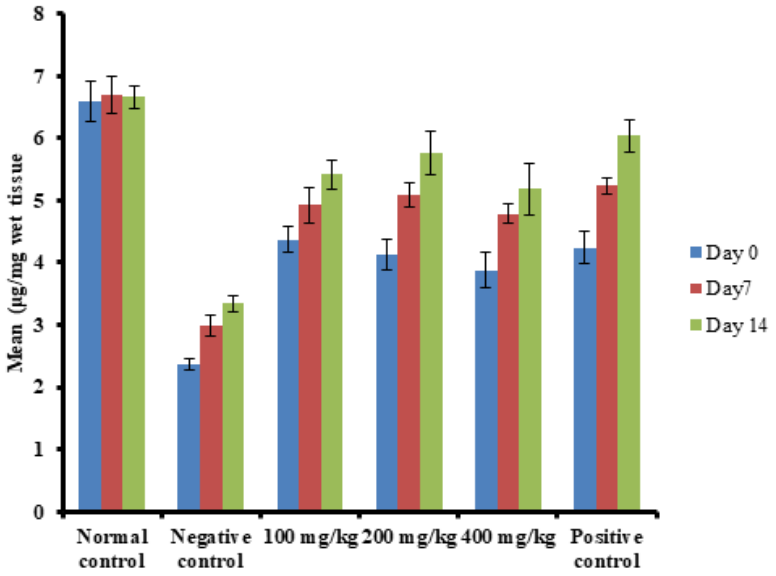

Fig 1: Effects of varying concentrations of the MEJS on Superoxide dismutase activity in PHZ induced anaemic rats.

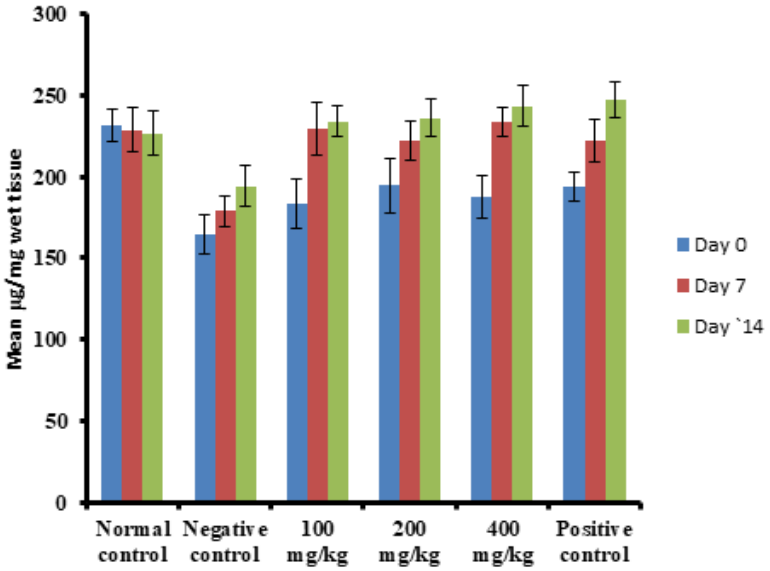

Fig 2: Effects of varying concentrations of MEJS on Catalase activity in PHZ induced anaemic rats.

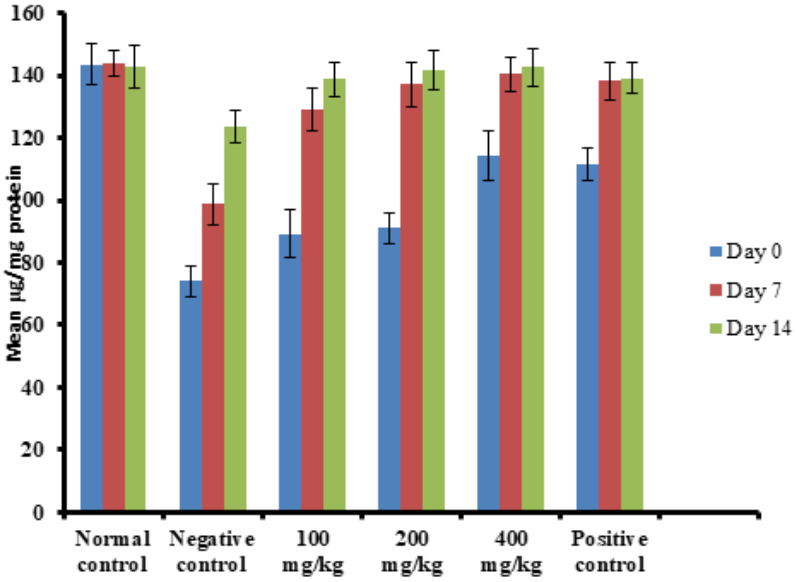

Fig 3: Effects of varying concentrations MEJS on Glutathione peroxidase activity in PHZ induced anaemic rats

Significant increase in plasma levels of MDA in all the induced groups was observed on day 0 compared to the normal control group, however, by day seven, a significant decrease ($p < 0.05$) in plasma levels of MDA and lipid peroxidation was observed in the treated groups compared to the untreated PHZ-induced group. At the end of the second week of treatment, there was no significant difference between the negative control group and the treated groups. However, the decline in the level of MDA was more in the extract, and ferrous sulphate treated groups (67%) than in the untreated anaemic rats (60%) (Figure 4).

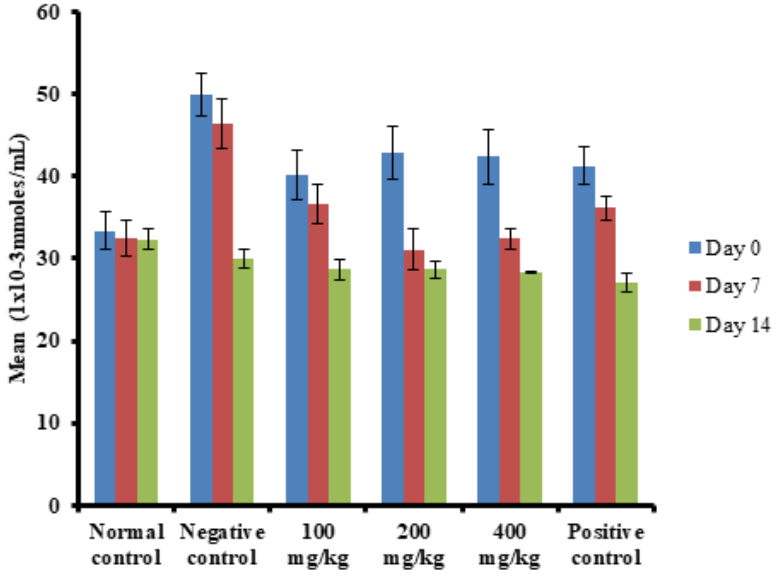

Fig 4: Effects of varying concentrations of MEJS on Malondialdehyde activity in PHZ induced anaemic rats.

DISCUSSION

Haematological effects

PHZ mainly causes hepatotoxicity, which leads to hemolytic anaemia by altering iron metabolism in the spleen, liver, duodenum and activates immune responses[22]. It equally increases the formation of reactive oxygen species, peroxidation of lipids and decreased glutathione levels[22,23]. PHZ-induced anaemia is characterized by decreased RBC count, HBG, HCT levels and a corresponding increase in MCV, MHC, and MCHC[23].

Following the induction of anaemia in this study, results obtained indicated that the rats developed acute hemolytic anaemia by presenting with decreased RBC count, HBG and HCT. These findings are consistent with those previously reported by Tariq (2006)[24], treating with thymoquinone and Baskaran and Suruthi (2016)[25], using *Kedrostis foetidissima* leaves where a significant decrease in Haematological parameters after administration of PHZ 60 mg/kg/day for two days was observed. The measurement of HBG, a component of RBCs, is the most direct indicator of clinical severity in hemolytic disease, and its monitoring is considered key in the management of hemolytic patients[26]. Administration of standard hematinic preparation and extract resulted in a significant increase in the numbers of RBC, HBG and HCT within the two weeks of treatment suggesting that MEJS has Anti-anaemic activity possibly by reversing the hematotoxicity induced by PHZ and/or activating the hemopoietic system. The improvement in hematologic indices recorded in the treated groups compared significantly with that observed in the normal control group, and there was no difference in the effects produced in the first week compared to the second week across the dose range. This suggests that the plant extract may have efficacy in the management of acute and not chronic anaemia. Hematinic effect against PHZ induced anaemia has been reported for *Justicia carnea*[27] a specie related to the plant under investigation.

The increase in WBC count in the induced groups compared to the normal control group can be attributed to the direct toxic effect of PHZ. When toxins enter the body, one of the ways they are combated by the immune system is the increased production of WBC[28]. The increase in WBC count in the test groups following induction was restored to normal by the first week and maintained through to the second week implying, recovery from the toxic effect of PHZ.

Thrombocytosis was observed in all the treated groups within the first week of treatment. Studies have shown that abnormal platelet counts are observed in individuals with anaemia[29]. A diphasic pattern of platelet response has been reported with thrombocytosis and thrombocytopenia in moderate and severe iron deficiency anaemia, respectively. Platelet counts may also be normal. In this case, thrombocytosis was observed one-week post-induction suggesting that moderate iron deficiency anaemia due to hemolysis was present in the PHZ-induced animals. By the second week, this was completely reversed, implying that the MEJS and ferrous sulfate may have the ability to reverse moderate iron-deficiency anaemia.

Haemolytic anaemia, as caused by PHZ, is known to precipitate Normocytic as well as Macrocytic forms of anaemia characterized by normal or large-sized red blood cells with normal or elevated MCV values respectively[29,30]. Normocytic anaemia due to hemolysis is characterized by normal or elevated RDW and thrombocytosis while Macrocytic anaemia due to hemolysis is characterized by normal or elevated RDW. Thus, the elevated platelet count, elevated MCV and elevated RDW levels following induction and treatment indicate macrocytic anaemia due to hemolysis. It equally suggests that the MEJS may have the ability to modulate these observed forms of anaemia at the tested doses.

Antioxidant effects

The induction of anaemia by PHZ resulted in a decrease in the antioxidant defence mechanism, which manifested as a decrease in antioxidant levels. This is in line with similar studies that observed that a plant with antioxidant activity would increase the levels of oxidative stress markers such as SOD, CAT, and GPX in the body [31,32] to ameliorate the effects of ROS in the biologic system.

According to Mittal and Kant[31], SOD

decreases with increased oxidative stress due to irreversible inactivation by its product (H_2O_2) and from the results, it was observed that PHZ induction resulted in reduced activity of the enzyme, but administration of the extract at different doses significantly reversed this reduction in comparison with the untreated induced group. Similarly, PHZ reduced the activity of Catalase and Glutathione peroxidase antioxidant activity, but the extracts significantly reversed this reduction compared to the negative control within the treatment period, suggesting that the extract may have the ability to ameliorate oxidative stress induced by PHZ.

MDA is a measure of lipid peroxidation of cell membranes. Lipid peroxidation, one of the major outcomes of free radical-mediated injury, damages membranes and generates several secondary products including aldehydes, such as MDA. During excessive oxidative stress, MDA increases and total antioxidant capacity decreases in the body[33,34]. Thus, a decrease in MDA implies an increase in total antioxidant capacity in the body, as seen in this study. Hence, it can be deduced from the results obtained that the plant extract may exact in-vivo antioxidant activity by reducing the generation of ROS which accounts for the decrease in lipid peroxidation and MDA production in the extract-treated groups.

CONCLUSION

The leaves of *J. secunda* have been shown in this study to improve *in-vivo* antioxidant status and reverse the hemato-toxicity induced by Phenylhydrazine in rats, thus providing scientific evidence to support its use in ethnomedicine as a blood booster.

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