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Ameliorative Potential of Desmodium adscendens Leave (EDAL) Extract on Blood Parameters in Phenylhydrazine-induced Anaemic Rats.

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Abstract - Medicinal plants, when regulated and used correctly can help to prevent and treat a variety of health conditions. We investigated the ameliorative potential of Desmodium adscendens leave (EDAL) extract on blood parameters in phenylhydrazine induced anaemic rats. Twenty-five (25) male albino Wistar rats weighing 180-200g, were randomly assigned to 5 groups of five rats each. Group1(normal control), Group 2 (anaemic control) was challenged with phenylhydrazine (60mg/kg, B.W., intraperitoneal) without treatment. Group 3 received EDAL orally at 150mg/kg, B.W., Group 4 and 5 were challenged with phenylhydrazine (60mg/kg, B.W,) and treated orally with 150mg/kg and 300mg/kg of extract of Desmodium adscendens leaves (EDAL) respectively. All animals were allowed free access to food and water pre and post treatment for 14 days. At the end of the treatment period, the animals were euthanized, and blood samples collected via cardiac puncture for biochemical analysis. The results showed that there was significant (P<0.05) decrease in most haematological indices except MCV, MCH, total white blood cell and neutrophils which recorded a significant (p<0.05) increase in anaemic control compared with normal control and extract group. Treatment with extract reverses these indices to almost similar level compared with control. Our findings suggest that the extract of Desmondium adscendens leaves has the capacity to ameliorates the Phenylhydrazine-induced haematotoxicity by reversing oxidative imbalances due to its phytochemical composition.

Keywords: Anaemia, Desmodium adscendens, haematological, Phenylhydrazine.

I. INTRODUCTION

Since time immemorial, man quest for survival and healthcare has led to the discovery of many plants of medicinal value before the advent of orthodox medicine. Although, its usage, particularly in Africa, was once believed to be primitive and wrongly challenged by foreign regions dating back to colonial rule in Africa and subsequently by the conventional or orthodox medical practitioners [1,2]. However, in the recent time, biomedical research has proven and authenticate the effectiveness of numerous medicinal plants for prevention, management and treatment of various ailments and diseases. Such diseases include anaemia and other blood-related disorders. Anaemia according the World Health Organization is a serious global public health problem that affect people, particularly, the pregnant women and Children in both developed and developing world. About 42% of children below 5 years of age and 40% of pregnant women are said to be anaemic [3]. Generally, it is estimated that anaemia affect approximately two billion people or one-third of the adult population globally [4,5]. Anaemia is of different types namely; haemorrahgic anaemia, aplastic anaemia, haemolytic anaemia and nutritional deficiency anaemia [6]. All incidents of anaemia are caused either by inherited disorders or environmental influence such as nutrition deficiencies, infectious diseases and exposure to drugs and toxins. In addition to adverse health consequences, the economic effects of anaemia on human capital results in the loss of billions of dollars annually [7]. Thus, Global nutrition targets endorsed by World Health Assembly (WHA), has set a goal of halving the anaemia prevalence by 2030, particularly among women of reproductive age [8]. To achieve this, multi-sectional actions are needed, and this may include, discovery of new therapeutic agents that are effective, cheap, and easily accessible.

Over the years, Phenylhydrazine (PHZ) has been used to experimentally induced anaemia in animal model. The haematoxicity effects of PHZ stem from its ability to distort oxidative balance via generation of Reactive oxygen species and lipid peroxidation of red blood cells [9,10]. This compromised the integrity of the cell membrane resulting in rapid haemolysis which led to anaemia of haemolytic type [5]. According to Sembulingam [6], anaemia of haemolytic type is the most common, and may arise from liver disease, renal disorder, hypersplenism, burns, parasitic and infectious disease like malaria and HIV/AIDS among others. Apart from its toxic effects on blood cells, exposure to Phenylhydrazine also cause damaging effects on other tissues such as the spleen, kidney and liver [11,12]. This could provide a suitable experimental model for study of anaemia in association with other diseases. Reports have shown that when anaemia is associated with another disease such has renal or hepatic diseases, it worsens the underlying disease symptoms, promote rapid disease progression and cause a poorer prognosis [13,14]. Hence, PHZ- induced anaemia can be used to investigate the haematinic effects of new therapeutic agents especially of plant origin [15,9]. *Desmodium adscendens* is one of the species in the *Desmodium* genus, it is a weedy perennial plant that commonly occurs in tropical areas of Africa, South America, Asia, Australia and Oceanis [16]. The plant is known with different names such as; Strong-back, beggarlice, pega pega and amer seco, hardstick, tick clover among others [17].

It is one of the numerous medicinal plants that has been used for various therapeutic purposes. It is believed to have been used for thousands of years by people native to the areas where it grows for a variety of health issues such as diarrheas, malaria, rheumatism, pneumonia, fever, epilepsy, asthma, jaundice, gastroduodenal ulcer, diabetes, sickle cell anaemia, hepatic diseases among others [18,19]. Numerous scientific studies [18,20] reported that the therapeutic phytochemicals in *Desmodium adscendens* includes; alkaloids (indolialkaloids, flavonoids (vitexin and isovitexin), saponins (Dehydrosoyaponin), polyphenols, tannin, anthrocyanin and astragalin. Rastogi *et al*., [17] listed that the main chemical found in amer seco are astragalin, beta- phenylethylamines, cosmosiin, cyaniding-3-0-sophoroside, dehydrosoyasaponins, hordenine, pelargonidin-3-0-rhaminoside, salsoline, soyasaponins, tectorigenin, tetrahydroionsquinolines and tyramine. These isolated compounds showed a wide spectrum of *in vitro* and *in vivo* pharmacological activities like anti-leishmanial, immunomodulatory, anti-asthmatics, anti-bacterials,anti- viral, cardioprotective, anti-inflammatory, hepatoprotective, smooth muscle relaxant and anti-oxidant activities, anti-ulcer, vermicidal [17,21]. However, Despite the fact that this plant has been extensively research, and its effect as a blood cleanser reported. There is paucity of report to buttress that claim. Therefore, this study was carried out to investigate the effect of ethanolic extract of *Desmodium adscendens* leaves on haematological parameters and some biochemical indices in Phenylhydrazine induced anaemic Wistar rats.

II. MATERIALS AND METHODS

Plant material and Drugs collection

Fresh *Desmodium adscendens* leaves was obtained from a farrow land in Bayside, Calabar South, cross River state and identified by a botanist in Department of Botany, University of Calabar, Nigeria.

Phenylhydrazine hydrochloride (Sigma-Adrich Chemical Company, St. louis, USA) was bought from BEZ pharmacy, Etagbor, Calabar.

Extract preparation

The extraction was done according to the method of Ekam and Udosen, [22]; the leaves of *Desmodium adscendens* were dried in an airy room for 7 days, away from direct sunlight. The dried leaves were grinded into a coarse powder. 113g of the powdered leaves were soaked in 800 ml of ethanol solution in a container and a stirrer was used to stir and mixed it thoroughly and kept for 72 hours.

The mixture was filtered with white cotton cloth, then the filtrate was further filtered with Whatman no 1 filter paper. The filtrate was evaporated to dryness under room temperature, and 16g of extract was collected into plain sample bottles and tag; Ethanolic Extract of *Desmodium adscendens* leaves (EDAL). This was stored in refrigerator prior to its use.

Experimental animals: A total of twenty-five (25) male Albino Wistar rats weighing 180-200g was used. They were allowed to acclimatize for two weeks under standard environmental condition and fed with rat chow and water *ad libitum*.

Experimental Design: The animals were randomly assigned into five (5) groups, and each group containing five animals were kept in wooden cages. The grouping was as follows; Group1 (normal control) receive only normal saline, Group2 (anaemic control) received intraperitoneal injection (I.P) of Phenylhydrazine (60mg/kg bwt) twice in 48hours. Group 3 (EDAL only) received 100mg/kg bwt of ethanoic extract of *Desmodium adscendens* leaves only, while Group4 (anaemic+ $EDAL_{LD}$) and Group5 (anaemic+$EDAL_{HD}$) were induced with anaemia as Group2 and then treated with 100mg/kg bwt and 200mg/g bwt of ethanolic extract of *Desmodium adscendens*(EDAL) respectively. All treatment except phenyl hydrazine (I.P) was given orally using oral cannula and the experiment lasted for 14 days.

All experimental procedures and animal handling were approved (343PHY301) by the Animal Research Ethics Committee of Faculty of Basic Medical sciences, University of Calabar, Calabar, Nigeria. The experimental duration was chosen based on previous studies, while the dosage of Phenylhydrazine and EDAL were based on the studies of Aribo *et al*., [23] and Ayoola *et al*., [24] respectively.

Collection of Blood samples: At the end of the administration, on day 15, after an overnight fast, the animals were anaesthetized in chloroform chamber. Blood samples was collected via cardiac puncture into plain sample bottles and EDTA sample bottles for biochemical and haematological analysis respectively.

Haematological Analysis: Blood samples were analyzed using automated haematology cell Counter (Counter Electric, Lutonbred Fordsbire Uk*).* The parameters determined were; Red blood cell (RBC) count, Packed cell volume (PVC), mean corpuscular volume (MCV), Haemoglobin (Hb)concentration, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), total white blood cell, Differential white blood cell count (neutrophils, eosinophils and basophils) and platelets.

Statistical analysis

Data were analyzed using a one-way analysis of variance (ANOVA) followed by a post hoc test (least square division. Results were presented as mean ± standard error of mean. Values at $P<0.05$ was considered statistically significant.

III. RESULTS

Haematological parameters in the different experimental groups

The results of the effects of EDAL on haematological parameters in different experimental group as presented in Table 1 shows significant decrease ($p<0.05$) in the mean value of total RBC count, Hb conc, PCV, and PLT in anaemic control compare to normal control, whereas, MCV, MCH, total WBC count and neutrophils were significantly increase($p<0.05$) when compared to normal control. While there was no significant different in MCHC, LYM, basophils and eosinophils relative to normal control and anaemic control. Treatment with EDAL in Group4(Anaemic+ $EDAL_{LD}$) and Group5 (Anaemic +$EDAL_{HD}$) record a significant improvement ($p<0.05$) in the blood indices compare with anaemic control. Also, EDAL only, significantly improve the haematological parameters compare to normal control.

Table 1: shows the effects of *Desmodium adscendens* on haematological parameters in different experimental groups.

Groups Parameters	Group1- normal control	Group2- Anaemic control	Group3- EDAL only	Group4- Anaemic+ $EDAL_{LD}$	Group5- Anaemic+ $EDAL_{HD}$
RBC (x10^6cells/ul)	8.09+0.25	3.87+0.22*	8.62+0.28[a]	6.15+0.38*[a b]	5.49+0.31*[ab]
Hb (g/dl)	14.30+0.22	9.32+0.44*	15.74+0.48[a]	13.88+0.32[a,b]	15.62+0.26*[abc]
PCV (%)	42.26+0.54	28.02+1.29*	46.44+0.79[a]	42.30+0.99[a,b]	47.06+1.08*[ab]
MCV (fl)	52.38+1.5	73.22+4.56*[b]	54.04+1.41[a]	69.59+4.13*[b]	86.63+4.90*[abc]
MCH (pg)	17.72+0.51	24.36+1.53*[b]	18.30+0.56	22.79+1.11*[b]	28.79+1.73*[abc]
MCHC (g/dl)	33.85+0.49	32.83+0.54	33.87+0.52	33.26+0.03	33.22+0.33
Total WBC count(x1000 cells/ul)	7.2+0.35	11.70+0.33*	8.40+0.40a	6.02+0.34[a,b]	10.26+0.738*[abc]

Neutrophils(%)	4.40+0.68	9.20+1.39*	6.40+1.24	7.48+1.36*[ab]	7.00+1.48[c]
Lymphocyte(%)	90.40+1.47	80.80+2.06*	93.60+1.17	87.60+197[a]	90.60+1.99[ac]
Monocyte (%)	2.00+0.55	0.20+0.20b*	3.00+1.30*	2.00+0.45[a]	2.75+0.56[a]
Esinophils(%)	1.00+0.02	0.10+0.10	0.09+0.04	1.12+0.10	0.09+0.0.2
Basophils	0.70+0.20	0.10+0.00	0.70+0.40	0.44+0.21	0.48+0.10
PLT(x10^3 cells/ul)	928.33+7.2	670.33+6.4*	930.00+10.1[*a]	766.67+10.3[a]	828.67+14.2*[bac]

Values are presented as mean+ SEM, n=5, *vs control at P<0.05; a=vs anaemic control at P<0.05; b=vs EDAL only at P<0.05; c=vs anaemic+ $EDAL_{LD}$ at P<0.05

IV. DISCUSSION

This present study demonstrated the ability of the ethanolic extract of *Desmodium adscendens* leaves (EDAL) to reverse haematological imbalances in phenylhydrazine induced anaemic rats. Apart from nutritional and idiopathetic factors, environmental insults arising from chemicals and drugs could predispose anaemic conditions in both human and animals. Phenyhydrazine (PHZ) is one of such drugs, and has been used over the years to experimentally induced anaemia in animal models.

Our results showed significant decrease in total RBC count, PCV, Hb conc., while MCH was increased following treatment with PHZ. This agreed with previous results [23,5]. Earlier reports have it that phenylhydrazine causes selective destruction of matured RBCs through oxidative stress [5,9]. PHZ generate more ROS, increase lipid peroxidation and decrease antioxidant capacity of the blood leading to oxidative stress [10]. More so the excess free radical could cause oxidative denaturation of Hb, impaired heme synthesis [25, 26] and compromised erythrocyte membrane stability resulting in anaemia of haemolytic type [5].

A remarkable increase in total WBC count arising from elevated level of neutrophils was observed. Although, this result contrast the study of Archibong *et al*. [11] who reported PHZ induced leucopenia. It could possibly be due to acute haemolysis demanding increased phagocytic capacity. Neutrophils are frontline phagocytic cells [27]. It could also be due to the body's attempt to increase its defense mechanism against PHZ-induced toxicity.

Administration of EDAL reverse the adverse effects of PHZ on erythrocyte indices, normalize leucocytes as seen in group4 and group5 of table 1. It is particularly interesting to note that the administration of EDAL in high dose produced even more favorable results compared with low dose. This Suggest a dose-dependent potency of EDAL to provoke strong erythropoietic response that upturn PHZ induced haematoxicity. It could also suggest that EDAL detoxify the blood which may enhanced the proper utilization of iron for synthesis of heme/haemoglobin for new red blood cells leading to improve Hb, and other erythrocytes indices. The detoxify ability of the extract could also explain the normalization of the white blood cells relative to anaemic control.

V. CONCLUSION

It is concluded that the ethanolic extract of *Desmondium adscendens* leaves (EDAL) has the ability to reversed haematoxicity induced by Phenylhydrazine toxicity.

Compliance with Ethical Guidelines
The rats were handled based on the 1985 guidelines of the National Institute of Health publication for laboratory animals. Ethical approval and guidelines on animal experiments were obtained from the University of Calabar Ethics Committee (Nigeria), and the approval number was 343PHY301.

Authors' Contributions
ESU and USU conceptualized the study. ESU, USU, and AAJ designed the study. AEU, USU, and contributed to the bench work. ESU, and AAJ provided expert advice and knowledge. All authors contributed to the development of the final manuscript and approved its submission. USU and AEU prepared the first draft, which was reviewed by ESU and AAJ.

Conflict of Interests
The authors declare that there is no conflict of interest.

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