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Original Research Article

Solanum macrocarpon Leaf Ameliorates Phenylhydrazine-induced Hemolytic Anemia, Hepatotoxicity and Oxidative Stress in Wistar Rats

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ABSTRACT

Several epidemiological studies have reported that the consumption and usage of plant food with bioactive content is associated with reduced incidence of pathologies. This study investigated the protective role of the aqueous leaf extract of *Solanum macrocarpon* against hemolytic anemia and liver toxicity caused by Phenylhydrazine in rats. The study was comprised of five groups (six albino rats each). Group 1 was normal control orally given normal saline once daily. Group II was an anemic group while Group III was orally given a daily dose of 300mg/kg *Solanum macrocarpon* aqueous leaf extract for 14 days. Group IV was induced with anemia and thereafter orally treated with 300mg/kg *Solanum macrocarpon* aqueous leaf extract for 14 days while Group V was induced with anemia and thereafter treated with 100mg/kg Ascorbic acid orally once daily for 14 days. Group II, IV, and V were intraperitoneally once daily induced hemolytic anemia with 50mg/kg Phenylhydrazine for three days consecutively. All rats were sacrificed on day 15. The observed significant reduction ($p<0.05$) in red blood cell (RBC), hemoglobin (Hb), packed cell volume (PCV), and Platelet count following Phenylhydrazine-induced anemia were attenuated after *Solanum macrocarpon* administration. Phenylhydrazine-induced anemia also increased significantly ($p<0.05$) aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), malondialdehyde (MDA), nitric oxide (NO) content with a concomitant decrease in catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx), all mitigated and reversed toward normalcy by *Solanum macrocarpon* leaf and Ascorbic acid corroborated by hepatic histopathological analysis. Thus, *Solanum macrocarpon* comparing favorably with ascorbic acid administration attenuated Phenylhydrazine-induced hemolytic anemia, toxicity, oxidative stress, and inflammation via mechanisms that involved its antioxidant and anti-inflammatory properties.

Keywords: Anemia, Hematology, Oxidative Stress, Phenylhydrazine, *Solanum macrocarpon.*

Introduction

According to the World Health Organization (WHO), a high population of the world (one-fourth) suffers from anemia and almost half of these sufferers occur in preschool-age children.[1] Red blood cells (RBCs) with decreased oxygen-carrying capacity in anemic sufferers clinically show weakness, blackouts, lightheadedness, lethargy, breathe shortness, dizziness, etc.[2] Hemolytic anemia is a normocytic anemia subtype, with low hemoglobin (Hb) level as a result of too much or premature RBC destruction.[2] One probable cause of diseases is exposure to environmental chemicals including Phenylhydrazine. Phenylhydrazine causes hemolytic anemia in research animals via RBCs cytoskeleton destruction.

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The believed mechanism of Phenylhydrazine-induced hemolytic anemia involves enhanced oxidative damage and oxidative stress in the membrane of RBC attributed to (i) membrane lipids peroxidation, (ii) generation of superoxide anion radical production, (iii) glutathione depletion due to hydrogen peroxide increased level in RBC (iv) other free radicals production including aryl and phenyl radicals.[3,4] Phenylhydrazine-induced hemolytic anemia mechanism of action also includes RBCs membrane rupture as a result of membrane proteins binding with phenylhydrazine.[3] Hemoglobin degradation resulting from conversion of oxyhemoglobin to methemoglobin by direct oxidation is also caused by phenylhydrazine.[5] Plants with medicinal benefits are of great importance to healthy living. Several plants continue to be examined against a wide variety of disorders. Epidemiological studies have established that vegetable and fruit consumption protect the body from oxidative stress and attack by scavenging free radicals. Natural antioxidants from plants including fruits and vegetables protect the body against oxidative stress thereby associated with disease prevention that has links to reactive oxygen species (ROS).[6] *Solanum macrocarpon*, a member of the family called Solanaceae popularly called garden egg is commonly served and eaten during ceremonies. It can be served together with kola or as a replacement for kola. It is also very useful in several delicacy preparations such as stew and salad. The leaves are always alternate, although sometimes opposite. The flowers of *Solanum macrocarpon* are star-shaped corollas showcasing five petals usually returned. Reports have it that *Solanum macrocarpon* leaf in Sierra Leone is used for throat treatment while the Kenyans use it for stomach problems[7]; In Nigeria, young fruits and leaves of *Solanum macrocarpon* serve as a vegetable while the fruits serve as laxatives and

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are used in heart problems treatment; the flowers and fruits play roles in teeth cleansing following chewing.[7] Several parts of *Solanum macrocarpon* including the fruit and leaf have been used for the management of constipation, and ulcers, tooth ache just as the leaf serves as a remedy for snake bites.[8] Diseases and infections of the skin as well as sores have also been managed using *Solanum macrocarpon* when applied to the infected areas.[9] It is also been reported that ailments such as asthma, catarrh, rheumatism, pains in joints, gastro-esophageal reflux disease as well as weight reduction have been well managed and treated using *Solanum macrocarpon*.[10] Phytochemical screenings of *Solanum macrocarpon* leaves detected saponins, flavonoids, alkaloids, terpenes, glycosides, and steroids as well as minerals with health benefits including calcium, magnesium, potassium, sodium, iron, and zinc [11]. *In vitro* free radical scavenging studies revealed higher antioxidant potency at higher concentrations, thus giving *Solanum macrocarpon* leaf free radical quenching potentials at higher concentrations.[11] This study is aimed at demonstrating the ameliorative potentials of *Solanum macrocarpon* leaf on Phenylhydrazine-induced hemolytic disorder, toxicity, and oxidative stress in albino rats.

Materials and Methods

Chemicals

Phenylhydrazine was sourced from Sigma Aldrich while diagnostic kits for biochemical measurements were purchased. All other chemicals and reagents were of analytical grade.

Collection, Identification, and Extraction of Solanum macrocarpon leaves.

Fresh leaves of *Solanum macrocarpon* were collected in December 2024 in a garden in Sapele Road, Benin City and authenticated by a taxonomist in the Department of Plant Biology and Biotechnology, Edo State University Iyamho, Nigeria. After being thoroughly washed, leaves were air-dried at room temperature (24 ºC) and crushed into fine powder. To prepare the aqueous extract, 1 kg of the powdered leaf was soaked in double distilled water (5000 ml) and placed for 48 hours at room temperature. Thereafter, filtration was carried out first through a Whatman filter paper No. 42 (125 mm) and subsequently cotton wool. The concentration of the filtrate was done using a rotary evaporator set at 40 ºC to one-tenth its volume and thereafter freeze-dried. An aliquot portion of the freeze-dried extract was weighed, dissolved in normal saline, and used for experiments.

Ethics Approval

The Edo State University Iyamho Institutional-Based Research (IBR) Grant committee gave approval and funding to this study. The guidelines for ethical conduct in the care and use of nonhuman animals in research as stipulated by the American Psychological Association (APA) were strictly adhered to.

Animals

Adult male Wistar albino rats (180-200 g) were purchased at Department of Zoology animal house, Ambrose Alli University, Ekpoma, Nigeria and kept in cages at the Animal house of Edo State University Iyamho maintained at room temperature (22±2 °C) with 12hour on/off light. The rats had access to rat feed pellets and water. They were acclimatized to laboratory conditions for five days before been divided into five groups.

Induction of Hemolytic Anemia and Experimental Design

Before the commencement of *Solanum macrocarpon* aqueous leaf extract treatment, the hemolytic disorder was intraperitoneally induced with 50mg/kg Phenylhydrazine daily for three consecutive days as previously described.[12,13] Rats with a packed cell volume (PCV) and hemoglobin (Hb) less than 35 % and 10 g/dL respectively were deemed anemic and included in this study. The five groups of the study are as follows: Group I (control): normal control (without treatment) was orally given daily saline once. Group II: anemic control (intraperitoneally administered 50mg/kg Phenylhydrazine once daily for three consecutive days only) and thereafter received normal saline orally throughout the study. Group III: non-anemic rats orally, once daily administered 300mg/kg *Solanum macrocarpon* leaf extract for 14 days consecutively. Group IV: anemic rats orally, once daily administered 300mg/kg *Solanum macrocarpon* leaf extract for 14 days consecutively. Group V: anemic rats orally, once daily administered 100mg/kg Ascorbic acid for 14 days consecutively.

The chosen 300mg/kg extract dosage followed an LD50 study of *Solanum macrocarpon* aqueous leaf extract which showed safety up to 5000 mg/kg. All administration was done between 9:00–10:00 am each day. All rats were sacrificed 24 hr. after the last extract treatment (day 15) via cardiac puncture/ocular vein and blood was collected in EDTA bottles (hematology) and plain tubes (serum) without the use of anticoagulant. The collected blood in plain tubes was centrifuged at 5000rpm after being allowed to stand for 1hr to obtain serum for biochemical analysis.

Tissue collection and preparation of homogenates

Immediately after the sacrifice, the liver was removed, rinsed with normal saline, and weighed. A portion of the collected liver tissues was fixed in 10% formalin (4% formaldehyde) for pathological assessment, while the remaining liver tissue was kept at –20°C for further analysis. A 10% liver homogenate was prepared using physiological saline, and the obtained supernatant was used for endogenous antioxidant and oxidative stress markers determination.

Biochemical Parameters

Assessment of liver function enzymes

Alanine Transaminase (ALT), Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), and Lactate Dehydrogenase (LDH) were carried out in blood with Randox commercially available kits according to manufacturer's instructions (Randox®, Randox labs, UK).

Hematological parameters

Hematological analyses such as Packed Cell Volume (PCV), Red Blood Cells (RBCs), White Blood Cells (WBCs), hemoglobin (Hb), and Platelets count were determined using a fully automated blood analyzer.

Assessment of lipid peroxidation and antioxidant activities in liver homogenates

Malondialdehyde (MDA) concentration, a lipid peroxidation product was quantified as stipulated by the method of **[14]** while endogenous antioxidants such as catalase (CAT) and Superoxide dismutase (SOD) assay were determined according to methods of [15] and [16] respectively. Reduced Glutathione (GSH) level was assessed using the method of [17] while Glutathione peroxidase (GPx) was carried out using the method of [18]. Nitric oxide (NO) was determined using the method of [19].

Histopathological analysis

Immediately after sacrifice, the liver of both the test and control rats were excised, and dried with blotting paper and a portion was instantly inserted in 10% phosphate-buffered formalin. Fixed tissue samples were placed in paraffin blocks and 5mm sections were prepared. Sections were hematoxylin and eosin (H&E) stained and examined using a light microscope. There was capturing of liver Photomicrographs.

Statistical analysis

Obtained data are expressed as mean ± standard deviation. Differences between the groups were determined using ANOVA and Turkeys' post hoc test using Graph Pad Prism software. Values are regarded as significantly different at $P < 0.05$.

Results and Discussion

The results of the hematological assessment (Table 1) revealed that rats given phenylhydrazine for three consecutive days at a daily dose of 50mg/kg experienced hemolytic anemia shown by a massive decline ($p < 0.05$) in the concentration of Hb, RBC, PCV, and platelets count in comparison with the normal and extract controls which were however significantly increased ($p < 0.05$) and attenuated towards normalcy following administration of *Solanum macrocarpon* aqueous leaf extract

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Table 1: Effects of *Solanum macrocarpon* aqueous leaf extract on Hematological parameters in Phenylhydrazine-induced anemic wistar rats

Treatment groups	Hb (g/dl)	WBC(x 10^9/L)	PCV (%)	RBC (x 10^{12}/L)	PLTS (x10^9/L)
Control	15.30^a±2.01	9.80^a±1.98	45.70^a±3.02	9.30^a±1.04	739.35^a±15.10
PHZ (anemic control)	6.30^b±1.32	14.90^b±1.99	19.10^b±2.13	2.40^b±0.45	532.20^b±10.50
S. macrocarpon (300 mg/kg) alone	20.30^a ±2.54	15.20^b±1.67	57.50^c±5.05	14.30$^{c\ c}$±1.74	634.50^c±12.70
PHZ + *S. macrocarpon* (300 mg/kg)	10.45^c±1.04	10.10^d±1.21	35.90$^{d.}$±2.01	5.50^d±0.67	625.10^d±15.60
PHZ + Ascorbic Acid (100mg/kg)	10.30^c±1.05	11.30^d±1.42	34.75^d±2.31	5.05^d±0.65	648.75^d±17.40

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$). PHZ: Phenylhydrazine, Hb: Hemoglobin, WBC: White Blood Cell; PCV: Packed Cell Volume; RBC: Red Blood Cell; PLTS: Platelets count

for fourteen days at a daily dose of 300mg/kg. The evaluation of hematological parameters such as the amount and form of RBCs, WBCs, PCV, Platelets and Hb levels is very important in toxicology and monitoring science as hematological indices reflect the majority of elements that influence the body's physiology, and variation from the global normal hematological ranges in animals indicates a specific physiological abnormality or a risk to physiological health.[20] In the present study as shown in Table 1, diagnostic of hemolytic anemia, Phenylhydrazine administration for three consecutive days at a dose of 50mg/kg caused a significant reduction ($p<0.05$) in the concentration of Hb, RBC, PCV, and platelet count and a significant increase ($p< 0.05$) in WBCs in line with previous findings.[21,22.23.24,25] Possible factors germane to the induction of anemia includes interference of phenylhydrazine with the synthesis of erythropoietin and accelerated erythrocyte destruction due to altered membrane permeability, increased mechanical fragility and/or malfunction in the metabolism of iron. [26]The observed decline in PCV, Hb, RBC and platelets counts due to Phenylhydrazine-induced anemia can also be ascribed to changes in the lipid composition of RBC membranes, which cause morphologically abnormal erythrocytes with decreased life span.[27] Our observed increase in WBCs can be attributed to the stimulation of the immune defense system" as literature has shown that an increased concentration of antigens in the body results in high values of WBC.[28] A crucial hormone called erythropoietin is produced by the kidneys and production of RBCs is regulated by erythropoietin. In renal disease, the kidneys are unable to produce enough erythropoietin, leading to a drop in the total blood cell count and subsequent anemia.[29] From this study, phenylhydrazine administration led to a significant decrease in the RBCs compared to the control and extract treated rats ($p < 0.05$), indicating that the kidneys cannot make enough erythropoietin to produce sufficient blood cells. Platelets are components of the blood with crucial role in blood clotting[30] and the reduction in platelet level in the Phenylhydrazine-induced anemic group in this study is an indication that that blood clotting time may be extended, which can lead to increased blood loss in injury cases.

Interestingly, following 14 days administration of aqueous leaf extract of *Solanum macrocarpon* or ascorbic acid to phenylhydrazine-induced anemic rats, Hb, RBC, PCV, platelet counts and WBCs were significantly improved towards normalcy similar to previous works of[21,24,25,31,32], an indication that *Solanum macrocarpon* contains bioactive compounds that enhance erythropoiesis, protects against phenylhydrazine-induced anemia, thus demonstrating anti-anemic effect of *Solanum macrocarpon* leaf. Happily, 14 days of *Solanum macrocarpon* treatment led to a significant increase ($p < 0.05$) in the RBCs showing its ability to significantly increase blood cell production. It is also possible that the 14 days administration of *Solanum macrocarpon* to Phenylhydrazine-induced anemic rats may have boosted hematopoiesis as *Solanum macrocarpon* leaf is known to contain several bioactive agents such as flavonoids, alkaloids, saponins as well as mineral nutrients including calcium, potassium, sodium, zinc and magnesium etc.[33] The presence of iron in the leaves of *Solanum macrocarpon* has also earlier been reported[33] and Iron is vital for the synthesis of red blood cells essential for formation of hemoglobin, the oxygen carrying pigment in red blood cells. Thus, iron availability in *Solanum macrocarpon* leaves may have modulated the regenerative response, anti-anemic property and replenish blood in the phenylhydrazine-induced anemic rats. Phytochemicals such as flavonoids are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow. Flavonoids have been reported to exert a positive influence on haematopoiesis.[34] Saponins are known to improve hematopoiesis by promoting survival through focal adhesion kinase and extracellular signal-regulated kinase activation and modulating cytokine production in the bone marrow.[35] The presence of saponin in the aqueous leaf extract of *Solanum macrocarpon* could thus play the same role in improving hematopoiesis and promoting the survival of blood cell lines. The observed significant increase ($p< 0.05$) in platelet count in *Solanum macrocarpon* extract treated group when compared to the anemic group indicate that the extract has a stimulating effect on platelet production. Platelets are responsive cells essential for the maintenance of vascular integrity and participate in homeostasis, thrombosis, and host immune responses. It was also observed that there was no significant difference between anti-anemic benefit of the plant extract and ascorbic acid.

Liver function assessment in the experimental animals investigated is presented in Table 2. The activities of liver function enzymes such as alanine aminotransferases (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and Lactate Dehydrogenase (LDH) were found to be significantly increased ($p < 0.05$) following Phenylhydrazine administration when compared with the control and *Solanum macrocarpon* extract animals. However, treatment of Phenylhydrazine-induced hemolytic anemic rats with *Solanum macrocarpon* extract at a daily dose of 300mg/kg or 100mg/kg Ascorbic acid for fourteen days significantly attenuated ALT, AST, ALP and LDH levels towards normalcy when compared with the Phenylhydrazine-induced hemolytic anemia administered rats. It was also observed that there was no significant difference between anti-anemic benefit of the plant extract and ascorbic acid. Among the various organs, the liver remains a primary site for metabolism of various chemicals and drugs. Liver function enzymes are commonly employed to identify inflammation and oxidative stress in liver health, as their levels are known to be caused by hepatocellular injury and/or impaired liver function. An increase in ALP levels in serum suggests damage or stress to the liver cell membrane. ALP is an enzyme associated with bile ducts and can rise when there's obstruction or damage to these ducts. Our observed significantly higher ($p < 0.05$) activities of ALT, AST and ALP seen in the serum of phenylhydrazine-induced anemic rats could be attributed to phenylhydrazine induced membrane damage resulting in the flow and leakage of these liver function enzymes into extra

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Table 2: Effects of *Solanum macrocarpon* aqueous leaf extract on liver function biomarkers in Phenylhydrazine-induced anemic wistar rats

Treatment groups	AST(U/L)	ALT(U/L)	ALP (U/L)	LDH (U/L)
Control	11.23^{a}±3.05	14.76^{a}±3.88	17.96^{a}±2.21	128.78^{a}±8.65
PHZ (anemic control)	48.31^{b}±3.01	49.34^{b}±2.23	52.64^{b}±3.87	606.24^{b}±15.52
S. macrocarpon (300 mg/kg) alone	13.24^{a}±2.06	13.28^{a}±2.90	19.43^{a}±2.76	120.07^{a}±10.54
PHZ + *S. macrocarpon* (300 mg/kg)	27.32^{c}±2.91	29.00^{c}±3.06	30.81^{c}±3.03	308.75^{c}±9.65
PHZ + Ascorbic Acid (100 mg/kg)	23.26^{c}±2.42	25.04^{c}±2.74	29.65^{c}±2.71	315.45^{c}±10.10

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$). PHZ: Phenylhydrazine; AST: Aspartate Aminotransferase; ALT: Alanine Aminotransferase; ALP: Alkaline Phosphatase; LDH: Lactate Dehydrogenase

hepatic circulation. With the pivotal functions of liver in maintenance, performance, regulating homeostasis of the body, xenobiotic metabolism, and detoxification and because the liver is the first site of contact for different types of therapeutic drugs, xenobiotics, chemicals, and toxins, this organ is vulnerable to chemical-induced injuries. Thus our observed elevated serum liver function enzyme levels shows hepatocellular damage and disruption of membrane integrity of the liver similar to previous studies.[36,37,38] However, the reversal towards normalcy of the activities of liver function enzymes by aqueous leaf extract of *Solanum macrocarpon* imply that the leaf extract has protective activity against phenylhydrazine induced hepatotoxicity by conferring stability on cell membranes thereby preventing leakage of the marker enzymes into blood circulation from the liver similar to previous protective studies.[36,37,38]

Also in this study as shown in Table 2, Phenylhydrazine caused significant increase ($p < 0.05$) in serum LDH activity similar to previous study[39] and this may be ascribed to hepatocellular necrosis as a result of the leakage of LDH into the blood stream.[40] However, the treatment of Phenylhydrazine-exposed hemolytic anemic rats with *Solanum macrocarpon* leaf extract at the dose of 300mg/kg significantly reduced ($p < 0.05$) the observed increase in serum LDH activity, an indication of the protective effect of *Solanum macrocarpon* which has been reported to be endowed with bioactive agents including flavonoids, saponins and alkaloids.[33]

The effect of Phenylhydrazine-induced anemia on liver antioxidant systems and MDA as well as NO formation in organs is illustrated In Table 3 and 4. Phenylhydrazine administration caused a significant increase ($p < 0.05$) in malondialdehyde (MDA) concentration while activities of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), reduced Glutathione (GSH) and Glutathione peroxidase (GPx) decreased significantly ($p < 0.05$) when compared with the normal control and *Solanum macrocarpon* extract treated animals .However, *Solanum macrocarpon* leaf extract was found to ameliorate the changes induced by phenylhydrazine on the biomarkers of lipid peroxidation and oxidative stress. The effect of *Solanum macrocarpon* extract on levels of Nitric Oxide (NO) in liver of Phenylhydrazine exposed rats is shown in Table 4. Phenylhydrazine elicited a significant increase ($p < 0.05$) in liver NO levels when compared with control and extract administered rats, and this increase was mitigated upon administration of 300mg/kg *Solanum macrocarpon* which restored the Phenylhydrazine-induced elevation of NO to near normal levels.

Hepatocytes are protected from free radicals and reactants by both enzymatic and non-enzymatic defense mechanisms including catalase (CAT), superoxide dismutase (SOD), Glutathione peroxidase (GPx) and reduced Glutathione (GSH), which react with and remove free radicals and ROS such as superoxide anion, hydrogen peroxide (H_2O_2), and hydroxyl radicals. [41]SOD catalyzes the dismutation of highly reactive superoxide anion which converts into O_2 and H_2O_2. CAT scavenges H_2O_2 and catalyzes its decomposition into H_2O and O_2.[42] GPx helps in reducing H_2O_2 and lipid peroxide levels, working alongside CAT to mitigate the harmful effects of hydroxyl radicals by limiting free radical production.[43] GSH donates electrons in these reactions, playing the role of a donor. In this study, Phenylhydrazine-induced anemic rats showed significant decreases ($p < 0.05$) in CAT, GSH, GPx, and SOD activities with a significant increase ($p < 0.05$) in MDA and NO contents compared to the *Solanum macrocarpon* extracts and control rats' groups similar to previous studies.[22,36,37,38,44] Endogenous antioxidants such as Glutathione system, CAT and SOD present in RBCs contribute heavily to blood antioxidant capacity.[45] However, these antioxidant capacities get compromised and altered, when there is anemia leading to attack and damage, oxidative stress and later acute hemolytic disorder. Our observed reduced level of SOD following phenylhydrazine administration reflects oxidative stress by eliciting production of free radicals and ROS thus leading to damage and toxicity. Also our observed decreased level of CAT, GPx and GSH following Phenylhydrazine administration led to free radicals and other ROS accumulation leading to oxidative stress and oxidative damage in Phenylhydrazine anemic rats. ROS and free radicals reacts with biological substrates which then leads to lipid peroxidation and subsequent impairment of functions of cell membrane as there will be decrease in fluidity of membrane as well as in activity change of enzymes and receptor that are membrane-bound.[46] MDA, a lipid peroxidation marker, associates with several negative effects such as increased membrane stiffness, osmotic fragility, and reduced mitochondrial longevity.[47] Consistent with our findings, Phenylhydrazine-induced anemia produced a significant rise ($p < 0.05$) in hepatic MDA level, an indicator of Phenylhydrazine-induced lipid peroxidation leading to tissue injury in the Phenylhydrazine anemic rats. The hepatic MDA rise shows failure of antioxidant defense system. It can be deduced that Phenylhydrazine-induced hemolytic anemia is attributable to free radicals and ROS production caused by Phenylhydrazine as revealed by high hepatic MDA and NO levels. It is known that produced free radicals enter circulation causing lipid peroxidation and then causing damage to skeleton of RBCs membrane, leading to oxidative damage, oxidative stress, hemolysis and anemia. However administration of *Solanum macrocarpon* leaf extracts to Phenylhydrazine induced hemolytic anemic rats enhanced antioxidant status towards normalcy similar to previous works[22,36,37,38,44] and attributable to ability of bioactive compounds in *Solanum macrocarpon* including flavonoids, saponins and alkaloids previously reported[33] to scavenge free radicals as well as by the extract ability to disable chain reactions and reduce damage.[48] The significant drop ($p < 0.05$) in SOD, CAT, GPx and GSH in phenylhydrazine anemic animals could be attributable to their increased usage. Their hepatic content was however recovered towards normalcy after 14 days of *Solanum macrocarpon* aqueous leaf extract administration, an action attributable to a decrease in hepatic peroxidative activity, which leads to a restoration of antioxidants levels. The administration of *Solanum macrocarpon* leaf extract led to significant reduction ($p<0.05$) in MDA compared to anemic control and thus, the modulation of lipid peroxidation, a good indication of the protective potential of *Solanum macrocarpon* leaf. The mechanism of this *Solanum macrocarpon* protection could also be the inhibition and scavenging of free radicals and ROS by suppressing cytochrome P450 bioactivation of phenylhydrazine to reactive metabolites. There was also no observed significance difference between anti-anemic and antioxidant benefit of the plant extract and ascorbic acid.

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Table 3: Effects of *Solanum macrocarpon* aqueous leaf extract on endogenous antioxidants in Phenylhydrazine (PHZ)-induced anemic wistar rats

Treatment groups	SOD (U/mg)	CAT (µmol/min/ml)	GSH (µM/mg)	GPx (U/mg)
Control	51.50^{a}±3.77	7.27^{a}±0.45	55.75^{a}±2.05	9.05^{a}±0.95
PHZ (anemic control)	23.50^{b}±2.59	0.47^{b}±0.21	31.90^{b}±2.00	3.54^{b}±0.87
S. macrocarpon (300 mg/kg) alone	96.65^{c}±6.50	7.85^{a}±0.35	58.55^{a}±3.01	10.18^{a}±1.01
PHZ + *S. macrocarpon* (300 mg/kg)	40.55^{d}±3.05	3.17^{c}±0.16	42.45^{c}±2.30	6.01^{c}±1.01
PHZ + Ascorbic Acid (100 mg/kg)	36.50^{d}±3.45	5.25^{d}±0.48	44.16^{c}±2.14	5.99^{c}±1.06

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$). PHZ: Phenylhydrazine; SOD: Superoxide dismutase; CAT: Catalase, GSH: Reduced Glutathione; GPx: Glutathione peroxidase.

Table 4: Effects of *Solanum macrocarpon* aqueous leaf extract on Lipid peroxidation in Phenylhydrazine-induced anemic wistar rats

Treatment groups	MDA (µmol/mg)	NO (µmol/g)
Control	1.15^{a}±0.21	90.31^{a}±5.01
PHZ (anemic control)	8.10^{b}±0.37	259.35^{b}±10.07
S. macrocarpon (300 mg/kg) alone	1.19^{a}±0.32	81.22^{a}±6.32
PHZ + *S. macrocarpon* (300 mg/kg)	3.75^{c}±0.53	156.73^{c}±7.75
PHZ + Ascorbic Acid (100 mg/kg)	3.63^{c}±0.46	162.21^{c}±5.04

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$). PHZ: Phenylhydrazine; MDA: Malondialdehyde, NO: Nitric oxide

Under normal situations, anti-inflammatory effect is produced by Nitric oxide (NO). However, in abnormal situations, NO serve as a pro-inflammatory mediator inducing inflammation as a result of overproduction by inducible nitric oxide synthase (iNOS).[49] Our observed increased level of NO in phenylhydrazine-hemolytic anemic rats can make NO react with superoxide anion forming poisonous nitrite anion and impairing function of the liver. However, this study observed that *Solanum macrocarpon* leaf extract is capable of decreasing excessive NO generation and the action of *Solanum macrocarpon* leaf extract in lowering NO was significant ($p<0.05$) at the explored dosage of 300mg/kg in comparison with the anemic control.

Histopathological examination of liver tissues of rats administered normal saline and *Solanum macrocarpon* leaf revealed normal rats lobular architecture with central veins and radiating hepatic cords that were normal (Figure 1 and 3), while three consecutive days of 50mg/kg Phenylhydrazine administration revealed hepatocytes with disordered arrangement, cellular swelling of hepatocytes, portal infiltrates of inflammatory cells, vacuolar degeneration, congestion, necrosis, Kupffer cells activation, cytoplasmic vacuolation as well as dilatation of bile duct and fibroplasia around the bile duct (Figure 2). However treatment of Phenylhydrazine-induced anemia and hepatotoxicity with *Solanum macrocarpon* leaf extract or Ascorbic acid remarkably and positively modulated these lesions and alterations (Figure 4 and 5).

Histopathological assessment of Phenylhydrazine-induced hemolytic anemic rats showed that three days consecutive administration of 50mg/kg Phenylhydrazine caused pathological lesions and alterations including necrosis in liver tissues which were however modulated and attenuated towards reversal, wellness and restoration by 14days administration of *Solanum macrocarpon* leaf extract possibly attributed to bioactive agents including flavonoids, saponins and minerals such as iron.

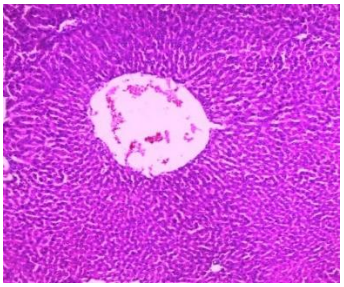

Figure 1: Photomicrograph of normal rat liver tissue showing the central vein with hepatocyte plates radiating from the central vein and separated by sinusoid

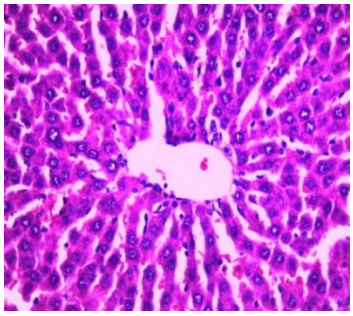

Figure 2: Photomicrograph of liver tissue of anemic rat that administered 50 mg/kg phenylhydrazine alone showing

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hepatocytes with disordered arrangement, cellular swelling of hepatocytes, portal infiltrates of inflammatory cells, vacuolar degeneration, congestion, necrosis, Kupffer cells activation, cytoplasmic vacuolation as well as dilatation of bile duct and fibroplasia around the bile duct.

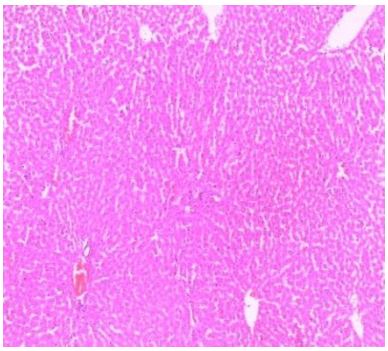

Figure 3: Photomicrograph of rat liver tissue that received only *Solanum macrocarpon* showing radiating hepatocytes from central vein separated by sinusoid.

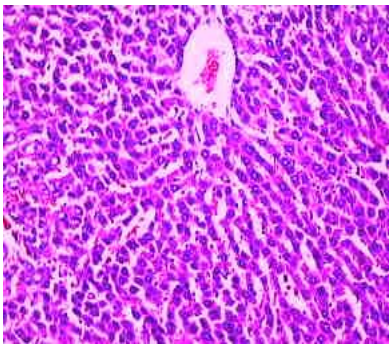

Figure 4: Photomicrograph of rat liver tissue that received 50mg/kg Phenylhydrazine and 300 mg/kg *Solanum macrocarpon* showing diminished necrosis, suppression of ballooning degeneration of liver cells as well as the number of vacuoles being significantly reduced.

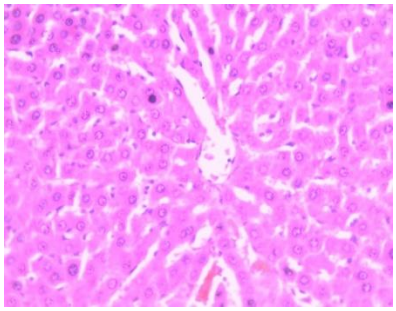

Figure 5: Photomicrograph of rat liver tissue that received 50mg/kg Phenylhydrazine and 100 mg/kg Ascorbic acid showing less inflammatory cell aggregates and improvement in hepatocyte homeostasis.

According to studies, *Solanum macrocarpon* leaves possesses minerals including Iron, zinc, magnesium, potassium, copper, calcium and sodium.[33] Calcium plays role in bone development and neurological function[50] while Sodium aids in control of fluids in the body as well as and preserve electric potential that exists in body tissue. Iron plays fundamental role in production of blood and in proper central nervous system operation[51] as well as ease with which lipids, proteins, and carbohydrates are oxidized. Magnesium helps regulate insulin release and blood pressure.[52] Thus *Solanum macrocarpon* leaf with its mineral concentration and phytochemicals would have rich nutritional value and antioxidant properties contributing to the hemopoietic stimulating and free radical quenching and scavenging actions of *Solanum macrocarpon* leaf extracts.

Conclusion

This study is the first on anti-anemic potency of *Solanum macrocarpon* leaves. The outcome of this study support consumption and usage of *Solanum macrocarpon* for health benefits including hematological related challenges, problems and anemia as well as supplement in our meals and diet in the management and prevention of oxidative stress-related pathologies and inflammation. *Solanum macrocarpon* leaf exerts this protective effect via attenuation of lipid peroxidation and oxidative stress by quenching free radicals and maintenance of antioxidant defense status.

Conflicts of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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References

1. Gallagher PG. Anemia in the pediatric patient. Blood 2022;140(6):571–593. DOI 10.1182/BLOOD.2020006479.
2. Baldwin C, Pandey J, Olarewaju O. 2022. Hemolytic anemia. Pathobiology of Human Disease: A Dynamic Encyclopedia of Disease Mechanisms 2022;115(6):1532–1543 DOI 10.1016/B978-0-12-386456-7.07907-7.
3. Nawaz H, Muhammad AS, Mohammad SI. Optimization of phenylhydrazine induced hyperbilirubinemia in experimental rabbit. Exp Anim. 2016; 65(4), 363–372.
4. Shukla P, Yadav NK, Singh P, Bansode FW, Singh RK. Phenylhydrazine induced toxicity: A review on its haematotoxicity. Int J. Basic Appl Med. Sci. 2012;2:86–91.
5. Nakanishi A, Kinuta K, Abe T, Araki K, Yoshida Y, Liang S, Li, SA, Takei K, Kinuta M. Formation of meso, N-diphenylprotoporphyrin IX by an aerobic reaction of phenylhydrazine with oxyhemoglobins. Acta Med. Okayama 2003;57:249–256.
6. Atawodi SE. Antioxidant potential of African medicinal plants. Afri J. Biotech. 2005;4:128-133.
7. Bukenya-Ziraba R, Bonsu KO. *Solanum macrocarpon* L. In: Grubben GJH, Denton OA (eds). Plant Resources of Tropical Africa. Vegetables/Legumes, Wageningen, Netherlands. 2004.
8. Oladiran JA. The effects of fruit color, processing technique and seed treatment on the germination of *Solanum macrocarpon* L. (Igbagba). Nig J. Tech Res. 1989;1(1):17–20.
9. Edijala JK, Asagba SO, Eriyamremu GE, Atomatofa U. Comparative Effect of Garden Egg Fruit, Oat and Apple on Serum Lipid Profile in Rats Fed a High Cholesterol Diet. Pak J. Nutr. 2005;4(4):245-249. http://dx.doi.org/10.3923/pjn.2005.245. 249.

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10. Dalziel JM. The useful plants of West Tropical Africa (New York: Longman) 1st edn. pp. 1937;433–435.
11. Usunobun U**,** Igwe VC. Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Solanum macrocarpon* leaves. Int J. Health 2016;4(1): 62 – 65.
12. Aziza AM, El-Shafey MM, El-Ezabi HHM, Ouda MM, Hegazy DSI. Effect of wheat germ oil on phenylhydrazine-induced toxicity in male albino rats. Egypt. J. Exp. Biol. *(Zool.)*, 2023;19(1):51 – 59 DOI : 10.5455/egysebz.20230108094930
13. Chinko BC, Pughikumo DT, Obia O, Udeh WC, Hart VO. Honey Attenuates Phenylhydrazine-Induced Hematotoxicity and Oxidative Stress in Male Wistar Rats. Int Blood Res & Rev. 2023;14(3):10-18.
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358. https://doi.org/10.1016/0003-2697(79)90738-3
15. Cohen G, Dembiec D, Marcus, J. Measurement of catalase activity in tissue extracts. Anal Biochem 1970;34:30-38. https://doi.org/10.1016/0003-2697(70)90083-7
16. Misra HP, Fridovich I. The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol Chem. 1972; 247: 3170-3175. https://doi.org/10.1016/S0021-9258(19)45228-9
17. Ellman GL. SH groups determination in biological fluids," Anal Biochem, 1970; 46:237–240.
18. Nyman M. Serum hemoglobin; methodological and clinical studies. Scand J. Clin and Lab Invest 1959;11:1-169.
19. Montogomery H, Dymock J. The determination of nitrite in water: colorimetric method of nitric oxide assay. Analyst, 1961;86**:** 414.
20. Nwachukwu DA, Uchendu IK, Nwafor GO, Ogbonna CC, Kwaor IA, Onyishi JC, Nwokwu OH , Jideofor GC, Okpara OM, Obinwa OR, Simon EO, Ewurum EC, Agbowu IB, Ishaq AO, Kalu PC, Ajibo VC, Ugochukwu IP, Okereke SE , Ojo UO. The Effects of Flauzifop-p-butyl on Behavioural Changes, Acetylcholinesterase, Serum Biochemical Parameters, and Hematological Indices in Albino Rats. Trop J Nat Prod Res. 2024;8(10): 8810 – 8818 https://doi.org/10.26538/tjnpr/v8i10.25
21. Soliman NA, Mansour SW, Ammar MA, Hassan NA, Mohamed RHA. Ameliorative Effect of Pomegranate Molasses on Phenylhydrazine-Induced Anemia in Rats. Trop J Nat Prod Res. 2024; 8(7): 7909 – 7914. https://doi.org/10.26538/tjnpr/v8i7.38
22. Kale OE, Awodele O, Akindele AJ. Protective effects of *Acridocarpus smeathmannii* (DC.) Guill. & Perr. root extract against phenylhydrazine-induced haematotoxicity, biochemical changes, and oxidative stress in rats. Biochem. Insights, 2019; 12:1178626419883243. doi: 10.1177/1178626419883243.
23. Amama EA, Udefa AL, Beshel FN, Archibong EA, Okpa S, Nwangwa JN, Felix NI, Akronyi G. Fresh palm oil improves impaired renal function in phenylhydrazine-induced anemic Wistar rats via its anti-anemic effect and modulation of expressions of pro-oxidant/antioxidants, inflammatory cytokines and caspase-3 in the kidneys. Phy. Pharma. 2022; 26:299-312. http://dx.doi.org/10.52547/phypha.26.3.8
24. Usunobun U, Inuumoru SC, Onyeaghor C, Lawuru D, Egbo H. Nephroprotective potential of *Chromolaena odorata* (L.) R.M. King & H.Rob. on Methotrexate-induced Kidney damage and Oxidative stress. J. Res Appl Basic Med Sci. 2022;8(3):118-127
25. Usunobun U, Okugbo OT. Anti-anemic, Anti-necrotic and Anti-fibrotic effect of *Vernonia amygdalina* post-treatment in Dimethylnitrosamine (DMN)-administered rats. Nig J. Pharma Appl Sci Res. 2016;5(2):1-7.
26. Yuan G, Dai S, Yin Z. Toxicological assessment of combined lead and cadmium: acute and sub-chronic toxicity study in rats. Food and Chem Toxic. 2014;65:260-268.
27. Stevens GA, Bennett JE, Hennocq Q. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. Lancet Glob Health 2015;3(09):e528–e536
28. Britton RS, Bacon BR. Role of free radicals in liver diseases and hepatic fibrosis. Hepatogastroenterology 1994;41:343-348
29. Pal P, Banerjee ER. Exploring the Effect of Phytochemical Extract (*Benincasa sp.*) on the Inflammatory Mediators and Regulatory Pathways of Acute Cisplatin Nephrotoxicity in Preclinical Mouse Model. Biomed. Res. Ther. 2024;11(6):6494-6510. DOI : 10.15419/bmrat.v11i6.896
30. Etim NN, Williams ME, Akpabio U, Offiong EE. Hematological parameters and factors affecting their values. J Agric Sci. 2014;2(1):37-47.
31. Airaodion AI, Ogbuagu EO. Consumption of tiger nut (*Cyperus esculentus L.*) improves hematopoiesis in Wistar rats. Int J. Res Rep in Hema. 2020;3(1):13-19.
32. Hassan HA, Hassan HA. The potential effect of tigernut oil on some haemato-biochemical blood indices in male albino rats. Egypt. J. Exp. Biol.(Zool.), 2007;3:49-54.
33. Usunobun U, Igwe VC. Phytochemical analysis, mineral composition and *in vitro* antioxidant activities of *Solanum macrocarpon* leaves. Int J. Health 2016;4(1):62–65.
34. Muriithi NJ, Maina GS, Maina MB, Kiambi MJ, Kelvin JK, Umar A, John MK, Ann NW, Piero NM, Eliud NNM. Determinants of hematological effects of methanolic leaf extract of *Vernonia lasiopus* in normal mice. J. Blood and Lymph, 2015;5(1):139
35. Chen X, Li B, Gao Y, Ji J, Wu Z, Chen S. Saponins from *Sanguisorba officinalis* improve hematopoiesis by promoting survival through FAK and Erk1/2 activation and modulating cytokine production in bone marrow. Front Pharmacol. 2017;8:130. doi: 10.3389/fphar.2017.00130.
36. Usunomena U, Okolie NP, Eze IG. Inhibitory Effect of *Vernonia amygdalina* on Dimethylnitrosamine (DMN)-induced Liver Fibrosis in Rats. Int J Clin Pharmacol Toxicol, 2015;4(4):179-184
37. Ikponmwosa BO, Usunobun U. Aqueous Leaf Extract of *Chromolaena odorata* Attenuates Methotrexate-Induced Hepatotoxicity in Wistar Rats. J. Fund Appl Pharm Sci. 2022;3(1):16-29.
38. Usunobun U, Osaigbovo JO, Okolie NP. Hepatoprotective Effect of *Raphiostylis Beninensis* Ethanol Root Extract on Carbon-tetrachloride (CCl_4)-induced Liver Attack and Damage in Rats. Am. J. Biomed. Sci. 2020;12(3),155-163; doi:10.5099/aj200300155
39. Usunobun U, Okolie NP. Synergistic, attenuative and modulatory activity in Dimethylnitrosamine (DMN)-induced fibrotic rats treated with *Vernonia amygdalina* and *Annona muricata* leaves. Nig J. Pharma Appli Sci Res. 2020;9(3):1-6
40. Kaptanoglu L, Kapan M, Kapan S. Effects of nimodipine and pentoxyfylline in prevention of hepatic ischemic damage in rats at normal and hypothermic conditions. Euro J. Pharma 2008; 587:253-256.
41. Mansourian M, Sadeghi H, Doustimotlagh AH. Activation of the glutathione peroxidase by metformin in the bile-duct ligation induced liver injury: in vivo combined with molecular docking studies. Curr Pharm Des 2018; 24(27):3256–3263.
42. Sharma V, Gupta R, Sharma S. Preventive Effects of *Tinospora cordifolia* extract against Aflatoxin-b1 induced oxidative stress in Swiss albino mice. Asian J Pharm Clin Res 2011;4(4): 149-155.
43. Gharu CP. Defense mechanism of natural antioxidants against free radicals. CAJMS. 2022; 3:163–170.
44. Bansode FW, Arya KR, Meena AK, Singh RK. Hematinic and anti-hematinic effects of the methanol extract of *Saraca*

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Indica stem bark against phenylhydrazine induced anemia in rats. World J. Pharm. Res., 2019; 8(9):1176-1201.
45. Fibach E, Rachmilewitz E. The role of oxidative stress in hemolytic anemia. Curr Mol Med. 2008;8:609-619
46. Onoja SO, Omeh YN, Ezeja MI, Chukwu MN. Evaluation of the in vitro and in vivo antioxidant potentials of *Aframomum melegueta* methanolic seed extract. J Trop Med 2014; 2014:159343.
47. Aydin S, Aral I, Kilic N, Bakan I, Aydin S, Erman F. The level of antioxidant enzymes, plasma vitamins C and E in cement plant workers. Clin. Chim. Acta 2004; 341:193–198. https://doi.org/10.1016/j.cccn.2003.12.007
48. Da Pozzo E, De Leo M, Faraone I, Milella L, Cavallini C, Piragine E, Filippi A, Braca A, Martinelli C, Bilia AR, Pistelli L. Antioxidant and antisenescence effects of bergamot juice. Oxid Med Cell Longev. 2018:9395804.
49. Cross RK, Wilson KT. Nitric oxide in inflammatory bowel disease. Inflamm Bowel Dis 2003; 9:179–189.
50. Isuosuo CC, Akaneme FI, Abu NE. Nutritional Evaluation of the Seeds of *Corchorus olitorius:* A Neglected and Underutilized Species in Nigeria. Pak J Nutr. 2019; 18:692-703.
51. Airaodion AI, Ayita EB, Oluba SO, Emaleku SA, Osunmuyiwa OJ. Chemical composition and nutraceutical potential of velvet tamarind fruit pulp. Asian J Biochem. Gene. Mol. Biol. 2021;3(5):22-31.
52. Balogun IO, Olatidoye OP. Chemical composition and nutritional evaluation of velvet bean seeds (*Mucuna utilis*) for domestic consumption and industrial utilization in Nigeria. Pak. J. Nutr., 2012; 11:116-122.

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