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Acute toxicity and effects of of *Solanum macrocarpon* leaf on Phenylhydrazine-induced haemolytic anaemia, blood glucose and Nephrotoxicity in Wistar rats

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Abstract

Background: A variety of toxic effects of the hydrazines have been described, including autoimmune disturbances in humans, human leukemogenesis, alterations in the liver, kidney, central nervous system.
Aim: This study aimed to determine whether *Solanum macrocarpon* aqueous leaf extract protect against Phenylhydrazine-induced hemato-toxicity and nephrotoxicity in anaemic male wistar rats.
Materials and Methods: Thirty adult male Wistar albino rats (180-200g) divided into five groups of six rats each were used for the study: Group 1 served as normal control and received normal saline orally once daily. Group II served as anaemic control while Group III received only 300mg/kg aqueous leaf extract of *Solanum macrocarpon* orally once daily for 14 days. Group IV rats were induced with anaemia and thereafter treated with 300mg/kg aqueous leaf extract of *Solanum macrocarpon* orally once daily for 14 days while Group V were induced with anaemia and thereafter treated with 100mg/kg Ascorbic acid orally once daily for 14 days. Group II, IV and V were intraperitoneally induced haemolytic anaemia with 50mg/kg Phenylhydrazine for three consecutive days. All rats were sacrificed 24 hr after last extract treatment.
Results: Acute toxicity study showed LD50 to be above 5000mg/kg as there was neither mortality nor toxicity observed up to the last dose of 5000mg/kg. Electrolyte concentration showed a significant decrease ($p<0.05$) in sodium ion and an increase in potassium ion concentration in Phenylhydrazine-induced anaemic rats which were however improved towards normalcy by *Solanum macrocarpon* leaf extract. *Solanum macrocarpon* significantly enhanced ($p<0.05$) Red blood cells, packed cell volume, haemoglobin and platelet count; significantly decreased ($p< 0.05$) levels of creatinine and urea as well as maintained blood glucose levels corroborated by histopathological findings
Conclusion: This study established *Solanum macrocarpon* leaf potential in modulating Phenylhydrazine-induced anaemia, electrolyte balance and nephrotoxicity in wistar rats.

Key words: Anaemia, Hematology, Nephrotoxicity, Phenylhydrazine, *Solanum macrocarpon*

Introduction

Anaemia is one of the commonest preventable causes of death in children under 5 years and in pregnant women[1] in poorer malaria-endemic countries, and hence, poses a great threat to global healthcare[2]. It has been documented that developing and underdeveloped countries have higher incidence of anaemia than in developed countries due to factors such as nutrient deficiency, recurrent infections and

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high prevalence of blood parasites like plasmodium and trypanosomes[3]. Anemia is characterized by three primary mechanisms: bleeding, reduced production of red blood cells, or increased breakdown of red blood cells. In this scenario, the main function of red blood cells is to convey oxygen from the lungs to various tissues in the body[4]. Therefore, any disorder that impacts the population, structure, or function of the red blood cells could be harmful to life[5]. Phenylhydrazine is one of the most potent carcinogens belonging to the hydrazine family of molecules[6]. Phenylhydrazine intoxication causes oxidative damage to erythrocytes[7] resulting in haemolytic anaemia with the involvement of spleen and liver[8]. It damages the cell membrane, producing gradual haematological alterations, inflammatory mediators and increased red cells apoptosis[9]. It also leads to hepatic and splenic iron overload[10]. Phenylhydrazine has been reported to cause lipid peroxidation in liver, kidney and spleen of mice[11]. Several types of iron, such as ferrous sulphate and ferrous fumarate have often been prescribed by orthodox medicine to cure anaemia[12]. However, numerous side effects including nausea, vomiting, abdominal pain, gastric discomfort, and constipation often accompany such treatments[13]. Therefore, naturally derived active compounds for treating anaemia have attracted global interest as a potentially beneficial alternative therapeutic option. A lot of vegetable resources in Nigeria and Africa remain neglected by research leading to the underdevelopment and underutilization of these resources for economic and environmental transformation. *Solanum macrocarpon* is a plant of the family Solanaceae like tomato, pepper and eggplant[14]. The leaves and young fruits of *Solanum macrocarpon* are prepared and eaten as a vegetable[15]. In Nigeria, the fruits are eaten as laxatives and are used in the treatment of heart disease while the flowers and fruits are chewed for cleaning the teeth[16]. In Kenya, the juice of boiled roots is drunk to get rid of hookworms while the crushed leaves are used to treat stomach disorders[16]. Phytochemical screenings of *Solanum macrocarpon* leaves detected the presence of flavonoids, alkaloids, saponins, terpenes, glycosides and steroids as well as minerals with health benefits including calcium, magnesium, potassium, sodium, iron, zinc[17]. In vitro antioxidant studies on *Solanum macrocarpon* leaves revealed that the higher the concentration, the higher the antioxidant activity, thus the better the free radical scavenging and quenching potentials[17]. The present study aims to assess the efficacy of *Solanum macrocarpon* aqueous leaf extract in mitigating phenylhydrazine-induced renal injury in male anaemic Wistar rats.

Materials and methods

Chemicals

Phenylhydrazine yellow powder was obtained 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 obtained in December 2024 from a local garden in Sapele road, Benin City, Edo State and authenticated by a taxonomist in the Department of Plant Biology and Biotechnology, Edo State University Iyamho, Nigeria. The Fresh leaves were thoroughly rinsed and air-dried at room temperature (24ºC) and then pulverized, crushed into fine powder using a manual blender and weighed. Aqueous extract was then prepared by soaking 1000g of the powdered leaf in 5 litres of double distilled water and kept at room temperature for 48hours (for thorough extraction). Thereafter, the extract was filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The filtrate was concentrated using a rotary evaporator set at 40ºC to one-tenth its original volume and then finally with freeze drier. The dried residue (crude extract) was stored at 4ºC. Aliquot portions of the crude plant extract residue was weighed and dissolved in normal saline for use on each day of experiments.

Acute toxicity and behavioural pattern studies

Acute toxicity study was carried out according to Organization for Economic Co-operation and Development (OECD) guideline[18]. Twenty-four male albino rats grouped into six consisting of four animals each was used for acute toxicity study. The animals were fasted overnight (i.e., away from food only; water was not withdrawn) prior to dosing.

Groups I–III received a single dose of 10, 100, and 1000 mg/kg b.w of *Solanum macrocarpon* aqueous leaf extract respectively, while groups V–VI received 1500, 3000, and 5000 mg/kg respectively. The rats were observed individually for the first 30 min with special attention being given in the first 4 h within 24 h and daily, thereafter, for the next 14 days for general behavioral, physiological, and pharmacological changes as well as lethality.

Induction of Haemolytic disorder (HD) and Toxicity

Before commencement of *Solanum macrocarpon* aqueous leaf extract treatment, haemolytic disorder was intraperitoneally induced with 50mg/kg Phenylhydrazine for three consecutive days as previously described.[19-21] Rats with a packed cell volume (PCV) and haemoglobin (Hb) less than 35 % and 10 g/dL respectively were deemed anaemic and included in this study.

Experimental design

Following the confirmation of Haemolytic disorder (anaemia) and toxicity, animals were distributed into five groups of six rats each. Adult male Wistar albino rats (180-200g) were obtained and housed in cages, under controlled temperature (22±2 °C) and 12-h on-off light schedule. They were fed with standard laboratory pellet and given free access to water. After acclimatization to laboratory conditions for five days, they were divided into the following groups:

- Group I (control): normal control (without treatment) received normal saline orally once daily.
- Group II: anaemic control (without treatment), but also received normal saline orally once daily.
- Group III: non-anaemic rats treated with 300 mg/kg of leaf extract of *Solanum macrocarpon* orally once daily for 14 days.
- Group IV: anaemic rats treated with 300 mg/kg of leaf extract of *Solanum macrocarpon* orally once daily for 14 days.
- Group V: anaemic rats treated with 100mg/kg Ascorbic acid orally once daily for 14 days

All administration was done between 9:00–10:00am each day. All rats were sacrificed 24 hr after last extract treatment (day 15) via cardiac puncture/ocular vein and blood collected in EDTA bottles (hematology) and plain tubes (serum) without the use of anticoagulant. The blood collected in plain tubes was allowed to stand for 45 min before being centrifuged at 5,000 rpm to obtain serum for biochemical analysis. The kidney were excised, rinsed with normal saline and weighed. A small portion of the collected tissues were fixed in 10% formalin (4% formaldehyde) for histopathological examinations.

Biochemical Parameters

Assessment of kidney function

Creatinine and urea were spectrophotometrically determined in serum using commercially Randox available kits according to the manufacturer's instructions (Randox®, Randox labs, UK).

Determination of blood Glucose Levels

The blood glucose levels of the rats were determined using One Touch Glucometer (Life Scan, USA) and Test Strips by. Blood glucose levels will be determined by collecting blood orbito rectally.

Measurement of Electrolytes

Serum concentrations of Na+, and K+ were determined using ion-selective electrolyte analyzer (Biolyte 2000/ BioCare Corporation, Hsinchu 300, Taiwan).

Haematological parameters

Hematological analyses such as haemoglobin (Hb), Red Blood Cells (RBCs), White Blood Cells (WBCs), Packed Cell Volume (PCV) and Platelets count were carried out using full automated blood cell counter PCE -210N.

Histopathological analysis

Immediately after sacrifice, the kidney of both the test and control rats were excised, dried with blotting paper and a portion instantly fixed in 10% phosphate buffered formalin. Fixed tissue samples were embedded in paraffin blocks and sections of 5 mm were prepared. Sections were stained with hematoxylin and eosin (H&E) and examined under Olympus/3H light microscope. Photomicrographs of the kidney were captured using a Moticam Images Plus 2.0 digital fitted to the light microscope.

Statistical analyses

Data obtained are expressed as mean ± standard deviation. Differences between the groups were determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test using Graph Pad Prism software. Values are regarded as significantly different at $P < 0.05$.

Results

The result of acute toxicity study showed that after oral administration of *Solanum macrocarpon* aqueous leaf extract, there was no mortality nor toxicity observed up to the last dose of 5000mg/kg showing that *Solanum macrocarpon* leaf is not toxic. Furthermore, no vari¬ance was observed in food intake and changes in body weight in normal and *Solanum macrocarpon*-administered rats. Using Lorke's approach, it was determined that the oral LD50 for *Solanum macrocarpon* aqueous leaf extract was greater than 5000 mg/kg, an indication of substantial level of safety as shown in Table 1. The *Solanum macrocarpon* administered rats had normal appearance without any signs of toxicity on the skin, fur, mucous membrane of the eyes, diarrhoea, tremors, or salivation.

Table 1: Acute Toxicity of *Solanum macrocarpon* aqueous leaf extract in Wistar Rats

Treatment groups	Treatment (mg/kg)	D/T	Observed Sign of Toxicity
1	10	0/4	-
2	100	0/4	-
3	1000	0/4	-
5	3000	0/4	-
6	5000	0/4	-

Key: D= death, T=Total number of animals used

The results on haematological assessment (Table 2) revealed that rats given phenylhydrazine for three consecutive days at a daily dose of 50mg/kg led to haemolytic anaemia as shown by massive decline in concentration of Hb, RBC, PCV and platelets count which were significantly increased ($p<0.05$) and attenuated towards normalcy following administration of *Solanum macrocarpon* aqueous leaf extract for fourteen days at a daily dose of 300mg/kg.

The results of renal assessment showed that there was a significant increase in levels of serum urea and creatinine following three days administration of 50mg/kg Phenylhydrazine in rats when compared to normal control and extract treated rats (Table 3). However, the observed increases in values of kidney biomarkers following Phenylhydrazine administration were abrogated towards normal levels in groups treated with aqueous leaf extract of *Solanum macrocarpon* as shown in table 3.

Table 2: Effects of *Solanum macrocarpon* aqueous leaf extract on Haematological parameters in Phenylhydrazine-induced anaemic wistar rats

Treatment groups	Hb (g/dl)	WBC ($x10^9$/L)	PCV (%)	RBC ($x10^{12}$/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

Table 3: Effects of *Solanum macrocarpon* aqueous leaf extract on kidney biomarkers in Phenylhydrazine-induced anaemic wistar rats

Treatment groups	Urea (mg/dl)	Creatinine (mg/dl)
Control	$14,86^a$±1.08	0.71^a±0.22
PHZ (anemic control)	41.01^b±2.11	2.40^b±0.16
S. macrocarpon (300 mg/kg) alone	15.18^a±1.12	0.81^a±0.04
PHZ + S. macrocarpon (300 mg/kg)	28.12^c±2.74	1.01^c±0.09
PHZ + Ascorbic Acid (100mg/kg)	27.23^c±2.32	1.03^c±0.08

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$). PHZ=Phenylhydrazine

Table 4: Effects of *Solanum macrocarpon* aqueous leaf extract on electrolytes in phenulhydrazine-induced anaemicwistar rats

Treatment groups	**K** (mmol/l)	**Na** (mmol/l)
Control	5.01^a±0.97	150.14^a±3.26
PHZ (anaemic control)	12.10^b±1.09	124.07^b±3.02
S. macrocarpon (300 mg/kg) alone	4.87^a±0.73	149.25^a±3.10
PHZ + S. macrocarpon (300 mg/kg)	8.24^c±1.00	137.11^c±2.09
PHZ + Ascorbic Acid (100mg/kg)	7.98^c±1.05	139.01^c±2.07

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$). K: Potassium ion; Na: Sodium ion. PHZ=Phenylhydrazine

The result of serum electrolyte concentration shown in Table 4 revealed a significant decrease in sodium ion and increase in potassium ion concentration in Phenylhydrazine-induced anaemic rats compared to normal control and extract treated groups. However, there was a significant increase ($P<0.05$) in serum

sodium ion concentration and decrease in potassium ion concentration in the *Solanum macrocarpon* treated phenylhydrazine-induced anaemic rats.

Also result of blood glucose concentration as shown in Table 5 revealed a significant increase in blood glucose concentration in Phenylhydrazine-induced anaemic rats which was however significantly decreased towards normal level in rats administered 300mg/kg *Solanum macrocarpon* or 100mg/kg Ascorbic acid.

Table 5: Effects of *Solanum macrocarpon* aqueous leaf extract on serum glucose level in phenulhydrazine-induced anaemicwistar rats

Treatment groups	Control	Phenylhydrazine (anaemic control)	*S. macrocarpon* (300mg/kg) alone	PHZ + *S. macrocarpon* (300 mg/kg)	PHZ + Ascorbic Acid (100mg/kg)
Glucose (mg/dl)	$94.77^{a}\pm2.06$	$129.87^{b}\pm3.02$	$91.55^{a}\pm2.73$	$101.92^{c}\pm2.84$	$103.21^{c}\pm2.52$

Values are expressed as Mean ± Standard Deviation. Values with different super scripts down the column differ significantly ($p<0.05$).

Histopathological examination of kidney tissues of rats administered normal saline and *Solanum macrocarpon* leaf revealed glomerulus, bowman's capsule and renal tubules with intact renal cortical and medullary basement membranes (Figure A and C), while three consecutive days of 50mg/kg Phenylhydrazine administration revealed glomerular degeneration and necrosis of renal tubular epithelium, interstitial nephritis and periglomerular inflammatory cell infiltration (Figure B). However treatment of Phenylhydrazine-induced anaemia and nephrotoxicity with *Solanum macrocarpon* leaf extract or Ascorbic acid positively modulated lesions and alterations (Figure D and E).

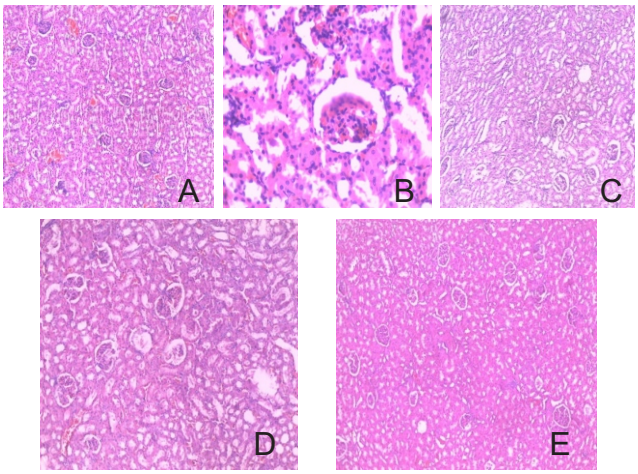

Plate A: photomicrograph of kidney sections of normal rat showing showing glomerulus, bowman's capsule and renal tubules with intact renal cortical and medullary basement membranes.

Plate B: photomicrograph of kidney of anaemic rats showing vacuolation, glomerular degeneration and necrosis of renal tubular epithelium, congestion of endothelial lining glomerular tuft, interstitial nephritis and periglomerular inflammatory cell infiltration.

Plate C: Photomicrograph of non-anaemic rat kidney treated with 300mg/kg *Solanum macrocarpon* alone showing glomerulus, bowman's capsule and renal tubules.

Plate D: Photomicrograph of phenylhydrazine-induced anaemic rat kidney treated with 300mg/kg *Solanum macrocarpon* showing marked improvement with less inflammatory cell aggregates,

Plate D: Photomicrograph of phenylhydrazine-induced anaemic rat kidney treated with 100mg/kg Ascorbic acid showing healing and tissue recovery.

Discussion

The acute toxicity study of *Solanum macrocarpon* aqueous leaf extract showed that the leaf does not possess any toxic effect at the studied dose levels and are safe beyond our studied highest dose level of 5000 mg/kg. Thus, result of the acute toxicity (LD50) study revealed that the LD50 value of *Solanum macrocarpon* is above 5000mg/kg body weight.

The proposed mechanism of Phenylhydrazine induced hemolysis involves increased oxidative stress in erythrocyte membrane caused by i) peroxidation of membrane lipids as a result of auto-oxidation of Phenylhydrazine and interaction of oxygen radicals with membrane lipids, ii) generation of superoxide anion radical by Phenylhydrazine and formation of Heinz bodies, iii) enhanced levels of hydrogen peroxide in erythrocytes resulting in glutathione depletion and iv) production of aryl, hydroxyl, superoxide, and phenyl radicals by Phenylhydrazine[22-23].

The kidneys are responsible for filtering waste products, and increased levels of these markers can suggest a decrease in their filtration capabilities. Observed decline in renal function evidenced by

significant increase in serum urea and creatinine in phenylhydrazine-induced anaemic rats was clearly seen in this study similar to previous findings[24.25,26-28]. Kidney damage is associated with decline in renal function which could lead to renal failure. Elevated urea and creatinine levels in the kidneys typically indicate impaired kidney function. An increase in serum urea indicate decrease in reabsorption at the renal epithelium while an increase in serum creatinine reflects impairment in the kidneys, particularly for glomerular filtration rate[29,30]. Thus, this study observed above normal, high concentrations of urea and creatinine in the blood of phenylhydrazine-induced anaemic rats compared to normal control and extract administered rats, which was corroborated by histopathological lesions is an indication of renal insufficiency, renal impairment and renal damage consistent previous works[24,25,26-28]. Excessive or chronic intravascular haemolysis leads to renal injury[31] and Phenylhydrazine-induced haemolytic anaemia can cause oxidative stress and oxidative damage thereby leading to nephron attack and kidney injury. However treatment of phenylhydrazine-induced anaemic rats with *Solanum macrocarpon* leaf extract for 14 days resulted in significantly reduced serum creatinine and urea concentrations, improved and restored renal function and physiologic features towards normalcy (Table 3), thus indicating that *Solanum macrocarpon* leaf offer reno-protection against Phenylhydrazine-induced anaemia similar to previous findings[24,25,26-28]. *Solanum macrocarpon* leaf proved its effective effect against Phenylhydrazine-induced nephrotoxicity as the leaf intake improved kidney function parameters; lessen impact of phenylhydrazine attack on kidney cells which is attributable to *Solanum macrocarpon* leaf antioxidants including flavonoids and free radical scavenging properties.

In the present study as shown in Table 2, diagnostic of haemolytic anaemia, phenylhydrazine administration for three consecutive days at a dose of 50mg/kg caused a significant reduction in the concentration of Hb, RBC, PCV and platelet count and a significant increase in WBCs in line with previous findings[24,25,32]. 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[33]. 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. Possible factors germane to the induction of anaemia include 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[34]. Interestingly, following 14 days administration of aqueous leaf extract of *Solanum macrocarpon* to phenylhydrazine-induced anaemic rats, Hb, RBC, PCV, platelet counts and WBCs were significantly improved towards normalcy similar to previous studies[32,35,36], an indication that *Solanum macrocarpon* contains bioactive compounds that enhance erythropoiesis, protects against phenylhydrazine-induced anaemia, thus demonstrating anti-anaemic effect of *Solanum macrocarpon* leaf. It is possible that the 14 days administration of *Solanum macrocarpon* to Phenylhydrazine-induced anaemic rats may have boosted haematopoiesis as *Solanum macrocarpon* leaf is known to contain several bioactive agents such as flavonoids, saponins as well as mineral nutrients including iron, zinc and magnesium[17]. Phytochemicals such as flavonoids are well known hemopoietic factors that have direct influence on the production of blood in the bone marrow.

The liver serves as a central metabolic hub, acting as the regulator of carbohydrate, protein, and lipid metabolism. A critical function of the liver is the storage and metabolism of glycogen as a readily available energy source. This process ensures glucose homeostasis during fasting periods, particularly for tissues with a preferential or obligate reliance on glucose, such as neurons and erythrocytes[37]. In this study, the significantly high serum glucose level seen in the phenylhydrazine-induced anaemic rats is an indication that Phenylhydrazine caused hyperglycaemia as it interfered with glucose metabolism (Table 5). However, our observed low blood glucose levels following *Solanum macrocarpon* leaf extract administration may be attributed to the antioxidant activity of *Solanum macrocarpon* leaf which

protects against β-cell damage induced by Phenylhydrazine similar to previous studies[38,39]. Changes in electrolyte levels can be linked to kidney function40. An elevation in potassium levels could signify decreased excretion[41,42]. In the present study there was hyponatremia and hyperkalemia in phenylhydrazine-induced anaemic rats similar to previous works[43,44] but however, *Solanum macrocarpon* leaf administration for 14 days significantly improved the serum concentrations of sodium and potassium levels towards normal.

Conclusion

The study highlights *solanum macrocarpon* leaf extract as a promising therapeutic agent for preventing renal damage, offering a natural and effective approach to mitigate the adverse effects of phenylhydrazine-induced toxicities. Thus, *Solanum macrocarpon* leaf can be useful in abrogating anaemic and renal function-related complications. Robust clinical studies are warranted to elucidate the efficacy and optimal dosage regimens of *Solanum macrocarpon* leaf in preventing haemolytic anaemia-induced renal damage as such research will facilitate the translation of promising preclinical findings into practical therapeutic strategies for safeguarding renal function in anaemic patients.

Ethics Statement

The study was carried out after approval by Edo State University Iyamho Institutional IBR Research committee and in accordance with the American Psychological Association (APA guidelines for ethical conduct in the care and use of nonhuman animals in research.

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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