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Nephroprotective Effects of *Mucuna Poggie* and *Telfairia Occidentalis* on Phenyl Hydrazine Induced Anaemic Albino Rats

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ABSTRACT

The chemical composition and blood boosting effects of leaf- extracts of *Telfairia occidentalis* and *Mucuna poggei* in phenyl-hydrazine-induced anaemia in Wister albino rats were investigated using standard procedures. A total of fifty four male albino rats were randomly assigned to 9 experimental groups of 1-9 (n= 6). Anaemia was induced in Group 2- 9 with 10 mg/Kg body weight of phenylhydrazine by administering a daily dose for four consecutive days, intraperitoneally. Group 1 (normal control) rats were administered with normal saline only. Group 2 (anaemic control) rats were left without treatment. Group 3 (standard control) rats were treated with standard multivitamin, groups 6, 5 and 4 were treated with 100, 200 and 400 mg/kg body weight of *Mucuna poggei* leaf-extract respectively, while groups 9, 8 and 7 were treated with100, 200 and 400 mg/kg body weight of *Telfairia occidentalis*, leaf-extract respectively by oral intubation for twenty one days. Results showed that PCV, Hb, RBC, MCV and MCH significantly ($P<0.05$) decreased in anaemic control rats relative to the normal control rats, but was significantly ($P<0.05$) increased on treatment with the standard multivitamin and ethanol leaf-extracts. Serum creatinine, urea and blood urea nitrogen levels significantly ($P<0.05$) increased in anaemic control relative to the normal control and significantly ($P<0.05$) decreased on treatment with the leaf-extracts and standard multivitamin. There was decrease but not significantly ($P>0.05$) in the level of calcium ion in anaemic control relative to the normal control. This was increased but not significantly ($P>0.05$) on treatment with the leaf-extracts and the standard multivitamin.Photomicrograph of the kidney induced with anaemia showed severe degeneration, atrophy and necrosis of the tissues which were all moderately healed with mild aggregate of inflammatory cells on treatment with the extracts and standard drug. This was consistent with the result of the biochemical parameters The results in this study indicated the blood boosting potentials of *Telfairia occidentalis* and *Mucuna poggei,* suggesting that the leaf-extracts of these plants may potentiate a strong nephroprotective and antianaemic effects.

Keywords:Nephroprotective, Antianaemic, *Mucuna Poggie*, *Telfairia Occidentalis* and Phenyl Hydrazine

INTRODUCTION

Plants provide an alternative in search for new drugs. There is a rich abundance of plants reputed in traditional medicine to possess protective and therapeutic properties [1,2,3]. A medicinal plant as defined by the world health organization

[4,5] is a plant which one or more parts of it contain substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [6,7]. Although modern medicine may be available in developing countries, herbal medicine is still popular in these countries till date. Plants have helped to maintain a relatively disease- free state when properly utilized as herbal medicine [8,9]. Herbs are also useful in the search for new drugs because they are valuable sources of new molecules which may be scientifically modified to provide improved drugs. Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economic development [10,11,12]. Anaemia is a condition in which the number of red blood cells or their oxygen-carrying capacity is insufficient to meet physiologic needs, which vary by age, sex, altitude, smoking, and pregnancy status. [13,14] definitions for anaemia differ by age, sex and pregnancy status as follows: children 6 months to 5 year anaemia is defined as a Hb level<11g/dl, children 5–11 years Hb < 11.5 g/dl, adult males Hb < 13 g/dl; non-pregnant women Hb <12g/dl and pregnant women Hb < 11g/dl. The primary cause of anaemia is iron deficiency, but a number of other conditions, such as malaria, parasitic infection, excessive consumption of drugs and other xenobiotics, other nutritional deficiencies, and haemoglobinopathies are also responsible, often in combination [15]. In 2002, iron deficiency anaemia (IDA) was considered to be among the most important contributing factors to the global burden of disease [16]. Anaemia is more prevalent in developing than in developed countries due to many factors such as poor nutrition, low socioeconomic status, high prevalence of blood parasites like plasmodium, trypanosomes and helminthes infestations. Primarily, iron deficiency may be high in developing countries where intakes may be low and blood sucking worms and malaria parasites may be high [17,18]. *Mucuna* is a genus of around 100 accepted species of climbing lianas (vines) and shrubs of the family Fabaceae: tribe Phaseoleae and typically found in Tropical forests. The leaves are tri-foliolate, alternate, or spiraled silky-pubescent beneath, the flowers are pea-like but larger, with distinctive curved petals, and occurring in racemes and the fruits are covered by itchy hairs that break loose on slight touching when fully dry in savannah woody and deciduous forest and secondary jungle [19] Like other legumes, *Mucuna* plants bear pods. They are generally bat-pollinated and produce seeds that are buoyant sea-beans. These have a characteristic three-layered appearance, appearing like the eyes of a large mammal in some species and like a hamburger in others (most notably *M. sloanei*) and giving rise to common names like deer-eye beans, donkey-eye beans, ox-eye beans, or hamburger seed [20]. *Telfairia occidentalis* (Fluted pumpkin) is one of the popular and widely grown vegetable crops in Nigeria particularly in the eastern (Anambra, Imo, Abia and Ebonyi States) and mid-western areas (Edo, and Delta States) and to an appreciable degree in the south western states (Ondo, Ogun, Ekiti, Oyo and Lagos). It is a pot-herb cultivated mainly for its succulent young leaves and shoots which are used as vegetables [21]. It is a high-climbing perennial with partial drought tolerance and parenting root system [22]. *Telfairia occidentalis* is a common homestead garden crop in southern Nigeria, mostly cultivated by women [23]. The crop is grown close to trees, walls, fences and structures on which the shoots are allowed to climb [24]. It could be allowed to creep on the ground or staked [25]. [26] recommended staking as the leaves of Telfairia spp are palatable and nutritious and are very much cherished by goats and it facilitates harvesting of the leaves and pods [27].

MATERIALS AND METHODS

MATERIALS

Animals

Fifty-four healthy adult male albino rats were used for the study. They were obtained from Dan Okoro farms, Abakaliki, Ebonyi State. The animals were allowed to acclimatise to the laboratory environment for a period of two weeks before the commencement of the experiment. They were kept in cages and were fed with water and standard pellet diet during the stabilisation period.

METHODS

Collection of Samples

Fresh matured leaves of *Telfairia occidentalis* and *Mucuna poggei* were harvested from a local farm in Okpoto, Ishielu local government area in Ebonyi state.

Plant Authentication

The plants were authenticated by a plant Taxonomist in the Department of Biotechnology, Evangel University Akaeze, Okpoto Campus Ebonyi State.

Extraction

Preparation of Leaf samples

The leaves were washed and air dried at room temperature for three weeks. After three weeks, dry samples were ground using mechanical grinder into powder form. The powder was sieved with the sieve of mesh size 1mm and then stored in polythene bags for further use.

Preparation of Leaf-Extracts of the Samples

The blended leaves were soaked in absolute ethanol in the ratio of 2:1 sample to solvent ratio and were allowed to stand for 48 hours Gray and Flatt, (2014). Each extracted solution was filtered off using a sieve cloth and Whatman No 2 filter paper (Cat no 1001 125) of pore size 125 mm. The filtrates were concentrated by distilling off the solvent and then evaporated to dryness on a water bath at 45 °C. The samples were then stored in refrigerator for subsequent usage.

Determination of Percentage Yield:

The extracts obtained from the two leaf samples from the two plants were weighed. The percentage yield per extract was calculated in terms of air dried weight of the leaf material as:

$$\text{Percentage yield} = \frac{\text{Amount of extract obtained}}{\text{Amount of initial sample}} \text{ X } 100$$

Experimental Design

A total of fifty - four male albino rats with body weight range of 170-210 g were randomly assigned to 9 experimental groups of 1-9 (n= 6). Anaemia was induced in Groups 2- 9 with phenylhydrazine by administering a daily dose of 10mg/Kg body weight for four consecutive days, intraperitoneally. Group 1 (normal control) rats were administered with normal saline only. Group 2 (anaemic control) rats were induced with anaemia and left without treatment. Group 3 (standard control) rats were treated with standard multivitamin. Groups 4 to 6 were treated with 400, 200 and 100mg/kg body weight of *Mucuna poggei* ethanol-leaf extract respectively, while Groups 7 to 9 were treated with 400, 200 and 100 mg/kg body weight of *Telfairia occidentalis* ethanol-leaf extract, respectively, by oral intubation. The animals in all groups were allowed free access to feed and water and the treatment lasted for twenty one days.

Induction of Anaemia

Anaemia was induced by the modified method of [28]. Anaemia was induced in Groups 2 - 9 with phenylhydrazine by administering a daily dose of 10 mg/Kg for four consecutive days, intraperitoneally making a total dose of 40 mg/Kg bwt. Anaemia was indicated by 40 % reduction in PCV, Hb and RBC counts values of all the anaemia-induced rat groups from the baseline values 4 days

after administration of phenylhydrazine. On the day of establishment of anaemia (day 5), the weight, Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC) concentrations and other haematological indices were taken in all the groups.

Collection of Blood Samples for Analysis of Haematological and Biochemical Indices.

At the end of the 21 days, after the last treatment, food was withdrawn. The rats fasted overnight with free access to water. They were then anaesthetized under chloroform vapor and sacrificed. A total volume of 3 ml whole blood was collected from each rat via cardiac puncture using sterile syringes and needles. The blood was divided into 2 portions. A volume of 1 ml of blood was collected into labelled sample bottles of Ethylene-Diamine-Tetra-acetic Acid (EDTA) for the haematological assay. The remaining 2 ml was emptied into plain tubes and allowed to clot for about two hours. The clotted blood was thereafter centrifuged at 3,000 rpm for 15 minutes to recover serum from clotted cells. The serum was used for biochemical analysis. Serum was separated with sterile syringes and needles and stored frozen until used for biochemical analysis. The internal organs, kidneys and liver were also surgically removed, weighed and stored forhistopathology.

Haematological Analysis

Haematological parameters were determined using BE10 Automated Haematology Analyser Midray, year 2020, China. Blood samples were collected through the cardiac puncture. Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC) concentrations and other haematological indices were determined in all the groups on day 5 to confirm anaemia and day 21 to determine the effect of treatment with extracts and the standard multivitamin (HS-12) on haematological parameters. These indices served as markers for the antianaemic effects of the ethanol leaf-extracts of the two plants.

Kidney Function Tests

Determination of Serum Creatinine Concentration

Procedure

Standard solution was prepared with 50 µl of standard and 500 µl of working reagent and mixed. After 30 seconds absorbance A1 of the standard was recorded. Exactly 2 minutes later, absorbance A2 was recorded using the spectrophotometer. Sample solution was prepared with 50 µl of sample (serum) and 500 µl of working reagent and mixed after 30 seconds absorbance A1 of sample was recorded. Exactly 2 minutes later, absorbance A2 was recorded using the spectrophotometer.

Calculation

$$\text{Serum Creatinine Conc.} = \frac{\text{change in Absorance of sample}}{\text{change in Absorbance of standard}} \times \text{concentration of standard}$$

Determination of Serum Urea Concentration.

Procedure

Standard solution was prepared with 10 µl of standard and 100 µl of reagent 1. Sample solution was prepared with10µl of sample and 100 µl of reagent 1 of all the samples. Both standard solution and sample solution prepared were mixed separately and incubated at 30 °C for 10 minutes. After the 10 minutes of incubation, reagent 2 and 3 were added to both the standard solution and sample solution for all the samples and mixed separately and incubated at 37°C for 15 minutes. After incubating for 15 minutes, absorbance of the sample (for all the samples) and standard against the blank wererecorded.

Calculation:

$$\text{Serum Urea Concentration} = \frac{\text{Absorance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard}$$

Determination of Blood Urea Nitrogen (BUN)

Blood Urea Nitrogen (BUN) was estimated by derivation from the formula:

$$\text{Blood Urea Nitrogen (BUN)} = \frac{\text{Molar mass of nitrogen}}{\text{Molar mass of urea}} \times \text{Serum Urea Concentration}$$

Where Molar mass of nitrogen = 28g
Molar mass of urea = 60g
Serum Urea Concentration is as determined for each rat in the study.

$$\text{Blood Urea Nitrogen (BUN)} = \frac{28}{\text{Molar mass of urea}} \times \text{Serum Urea Concentration}$$

Determination of Serum Calcium.

Procedure

Three test tubes labelled reagent blank, standard and sample were set up. To the test tube labelled reagent blank was pipette 25 µl DDH_20 (EDTA solution), to the test tube labelled standard was pipette 25 µl of the Standard, to the test tube labelled Sample was pipette 25 µl of the sample. Then to each of the three test tubes labelled Reagent blank, Standard and Sample were pipette 1000 µl of the working reagent. The contents in each of the three test tubes were mixed and incubated for 5- 50 minutes. Thereafter, the absorbance at 578 nm of the standard and sample against the reagent blank were read.

Calculation:

the wax which solidified when the spatula was removed.
(vii) Microtomy: the block of tissues was sectioned using rotary microtom; it was trimmed to obtain the cutting surface of the tissue at 15 micron and was sectioned at 5 micron and dried in hot plate for staining.

Haematoxylin/Eosin (H/E) Method of Staining

Procedure

The Paraffin wax block of tissue which was sectioned, trimmed and dried was dewaxed in xylene for 30 minutes. Xylene was then removed by rinsing in absolute alcohol, 90 %, 70 % and 50 % alcohol for two seconds each. It was then washed in 2 changes of water and stained in haematoxlyin for 20 minutes. Again it was washed in water and differentiated in 1 % acid. It was then blued in tap water and washed in water again. It was then counter stained in Eosin for 5 minutes, washed in water, dried and cleared in xylene. Finally it was mounted in D.P.X and dried for micrography and interpretation.

Statistical Analysis

The results were expressed as mean ± standard deviation (SD). The data wer subjected to One Way Analyses of Variance (ANOVA) by Turkey Post hock test. The data werey analysed using computer software known as Graph Pad Prism 7. Values of P less than 0.05 ($P<0.05$) were considered to be statistically significant.

RESULTS

Kidney Function Parameters

Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Creatinine concentration of phenylhydrazine-induced anaemia in Wistar rats.

Serum creatinine level significantly ($P<0.05$) increased in anaemic control relative to the normal control, but was significantly ($P<0.05$) decreased on treatment with the leaf-extracts and the standard multivitamin in a dose dependent manner (Figure 1). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly ($P<0.05$) lower level of Serum creatinine at the 400 mg/Kg (MP1) and the 200 mg/Kg relative to the leaf-extracts of *Telfairia occidentalis* (TO1) and (TO2) respectively and was similar to the normal control (NC) and the standard control (SC) (Figure 1). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a

significantly (P<0.05) lower level of Serum creatinine at the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO3). MP3 was similar to the normal control (NC) and the standard control (SC) (Figure 1).

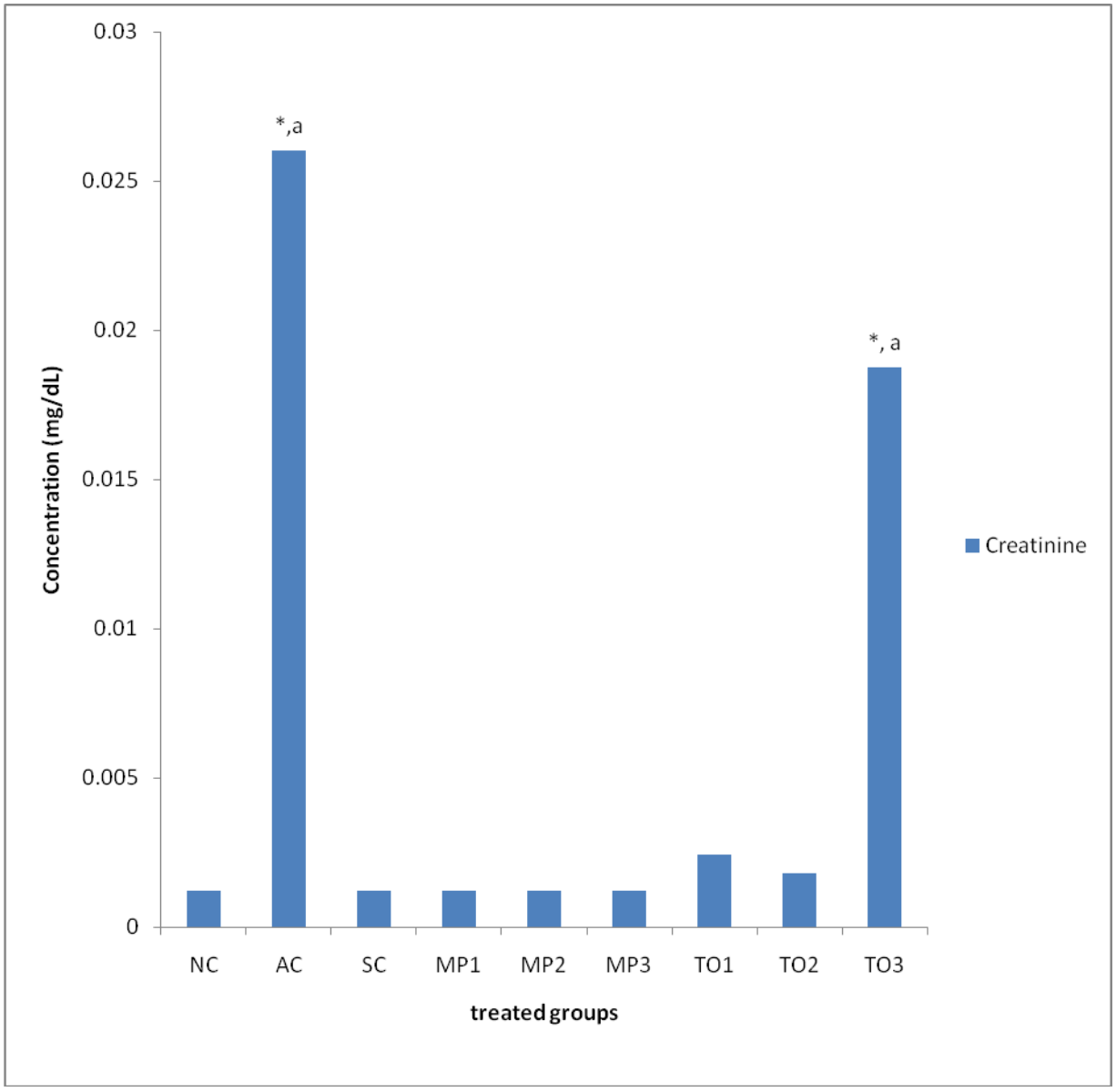

Figure 1. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Creatinine concentration of phenylhydrazine-induced anaemia in Wistar rats.
*Significantly different vs NC. [a]Significantly different compared to treatment groups. Mean±SD (n=replica of 5). NC-Normal control, AC- Anaemic control, SC- Standard control, MP1, 2 and 3. *M. poggei* at varying concentrations, TO1, 2 and 3. *T. occidentalis* at different concentrations.

Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Urea concentration of phenylhydrazine-induced anaemia in Wistar rats.

Serum Urea level significantly (P<0.05) increased in anaemic control relative to the normal control, but was significantly (P<0.05) decreased on treatment with the leaf-extracts and the standard multivitamin (Figure 2).

The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) lower level of Serum Urea at the 400 mg/Kg (MP1), 200 mg/Kg (MP2), and the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO1), (TO2) and (TO3) respectively and was similar to the standard control (SC) but higher than the normal control (NC). The levels of Serum Urea at the 400 mg/Kg (MP1), 200 mg/Kg (MP2), and the 100 mg/Kg (MP3) were dose dependent (Figure 2).

The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) lower level of Serum Urea at the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO3). MP3 was significantly (P<0.05) higher than the normal control (NC) and the standard control (SC) (Figure 2).

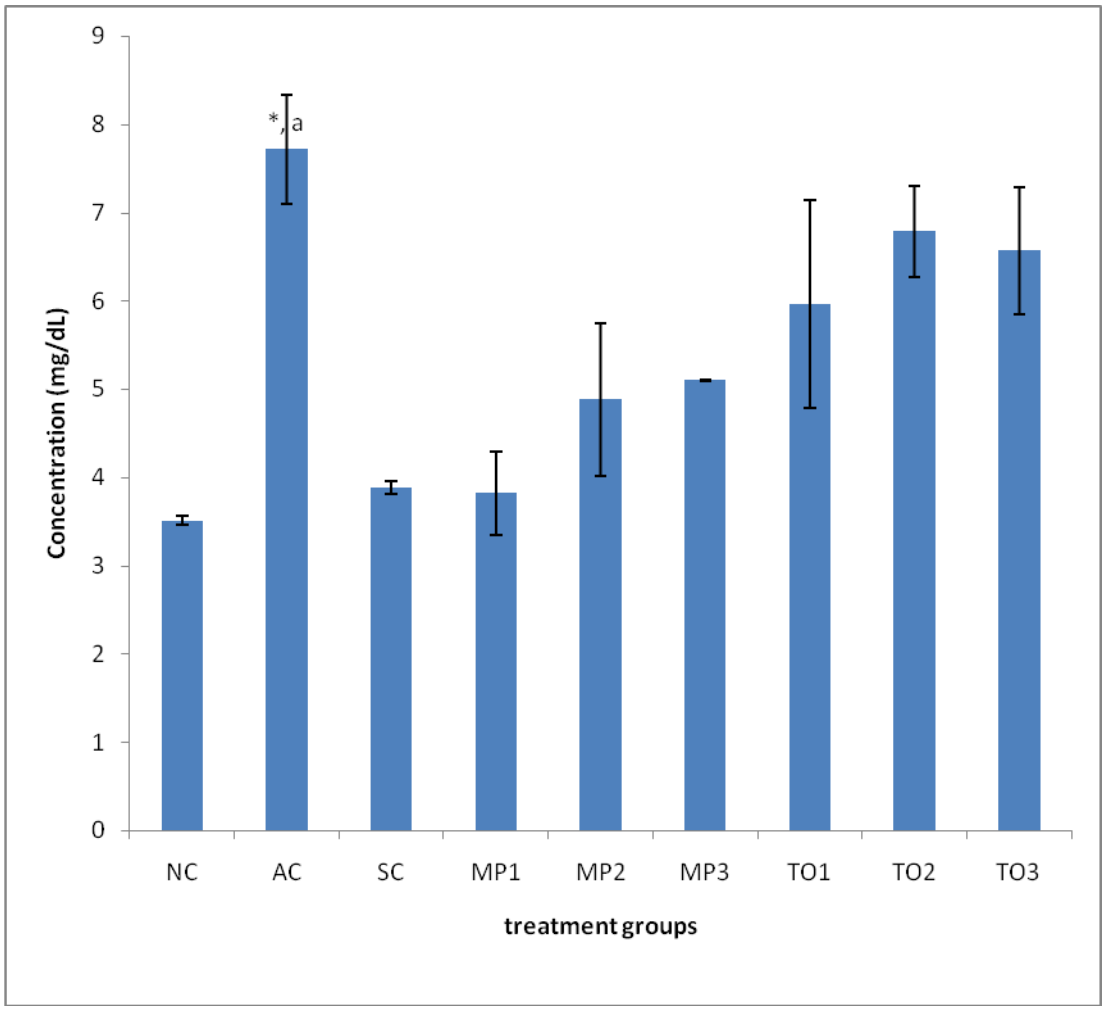

Figure 2. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Urea concentration of phenylhydrazine-induced anaemia in Wistar rats. *Significantly different vs NC. [a]Significantly different compared to treatment groups. Mean ± SD (n=replica of 5). NC-Normal control, AC- Anaemic control, SC- Standard control, MP1, 2

and 3. *M. poggei* at varying concentrations, TO1, 2 and 3. *T. occidentalis* at different concentrations.

Effect of ethanol leaf-extracts of *Mucuna poggei* (MU) and *Telfairia occidentalis* (TO) on Blood Urea Nitrogen (BUN) concentration of phenylhydrazine-induced anaemia in Wistar rats.Serum Blood Urea Nitrogen (BUN) level significantly ($P<0.05$) increased in anaemic control relative to the normal control, but was significantly ($P<0.05$) decreased on treatment with the leaf-extracts and the standard multivitamin in a dose dependent manner (Figure 3). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly ($P<0.05$) lower level of Serum Blood Urea Nitrogen (BUN) at the 400 mg/Kg (MP1), 200 mg/Kg (MP2), and the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO1), (TO2) and (TO3) respectively and was similar to the standard control (SC) but significantly ($P<0.05$) higher than the normal control (NC). The levels of Serum Blood Urea Nitrogen (BUN) at the 400 mg/Kg (MP1), 200 mg/Kg (MP2), and the 100 mg/Kg (MP3) were dose dependent (Figure 3). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly ($P<0.05$) lower level of Serum Blood Urea Nitrogen (BUN) at the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO3). MP3 was significantly ($P<0.05$) higher than the normal control (NC) and the standard control (SC) (Figure 3).

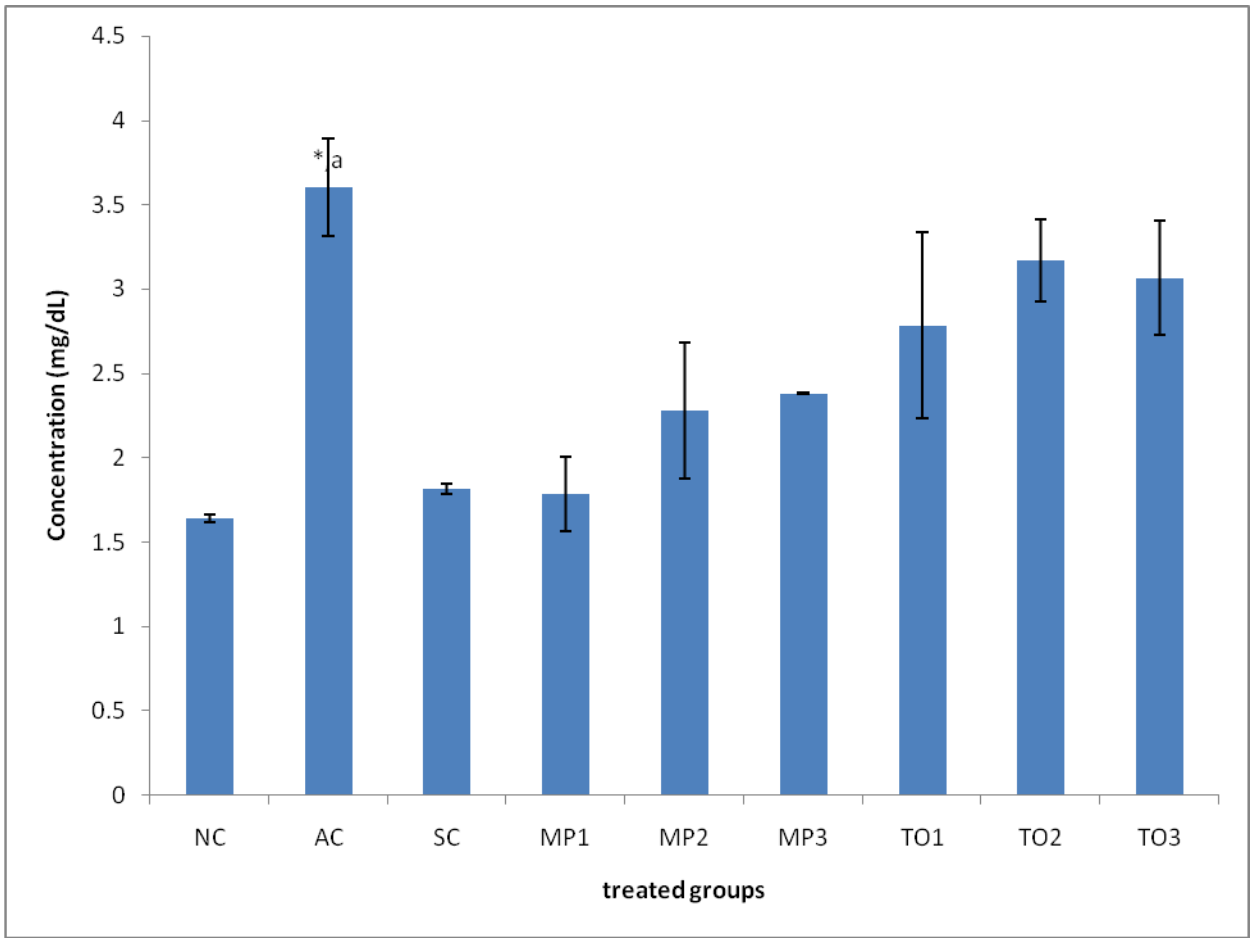

Figure 3. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on Blood Urea Nitrogen (BUN) concentration of phenylhydrazine-induced anaemia in Wistar rats.

*Significantly different vs NC. [a]Significantly different compared to treatment groups. Mean±SD (n=replica of 5). NC-Normal control, AC- Anaemic control, SC- Standard control, MP1, 2 and 3. *M. poggei* at varying concentrations, TO1, 2 and 3. *T. occidentalis* at different concentrations.

Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Calcium concentration of phenylhydrazine-induced anaemia in Wistar rats.There was decrease but not significantly (P>0.05) in the level of Ca^{2+} ion in anaemic control relative to the normal control. This was increased but not significantly (P>0.05) on treatment with the leaf-extracts and the standard multivitamin drug (Figure 4).

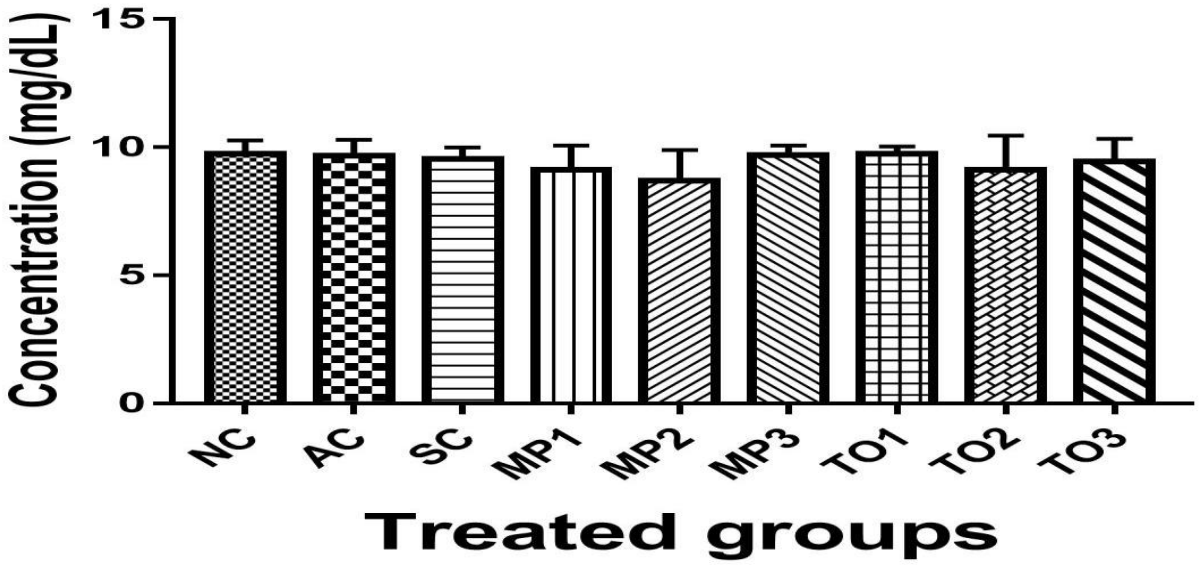

Figure 4. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Calcium concentration of phenylhydrazine-induced anaemia in Wistar rats. n=5 mean ± SD *(p > 0.05).* NC-Normal control, AC- Anaemic control, SC- Standard control, MP 1, 2 and 3. *M. poggei* at varying concentrations, TO1, 2 and 3. *T. occidentalis* at different concentrations.

Histopathology

Histopathology of the kidney

Photomicrograph of the kidney induced with anaemia showed severe degeneration, atrophy and necrosis of the tissues which were all moderately healed with mild aggregate of inflammatory cells on treatment with the extracts and standard drug. This was consistent with the result of the biochemical parameters. Photomicrograph of 1 control section of kidney (X400)(H/E) shows well perfussed normal renal architecture with glomeruli (G) , bowman space (BS), renal tubules (RT)and tubular cell (TC) well outlined Photomicrograph of 2 section of kidney indused with Anaemia show moderate to severe the renal tissue with severe aggregate of intra renal imflammation (IRI) , moderate glomerular attrophy (GA) and moderate tubular necrosis (TN). Photomicrograph of 3 section of kidney indused with Anaemia and treated with standard drug (X400(H/E) show moderate healing with mild aggregate of renal inflammatory cell (ARI)arround the glomeruli. Photomicrograph of 4 section of kidney indused with Anaemia and treated with 400mg/kg of MU1 (X400(H/E) show moderate healing with mild aggregate of intra renal inflammation (ARI) . Photomicrograph of 5 section of kidney indused with Anaemia and treated with 200mg/kg of

MU1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) Photomicrograph of 6 section of kidney indused with Anaemia and treated with 100mg/kg of MU1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) and moderate tubular necrosis (TN). Photomicrograph of 7 section of kidney indused with Anaemia and treated with 400mg/kg of TO1 (X400(H/E) show moderate healing with mild aggregate of intra renal inflammation (ARI) around the glomeruli. Photomicrograph of 8 section of kidney indused with Anaemia and treated with 200mg/kg of TO1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) around the atrophied (A) glomeruli. Photomicrograph of 9 section of kidney indused with Anaemia and treated with 100mg/kg of TO1 (X400(H/E) show mild to moderate healing with mild aggregate of intra renal inflammation (ARI) around the atrophied (A) glomeruli.

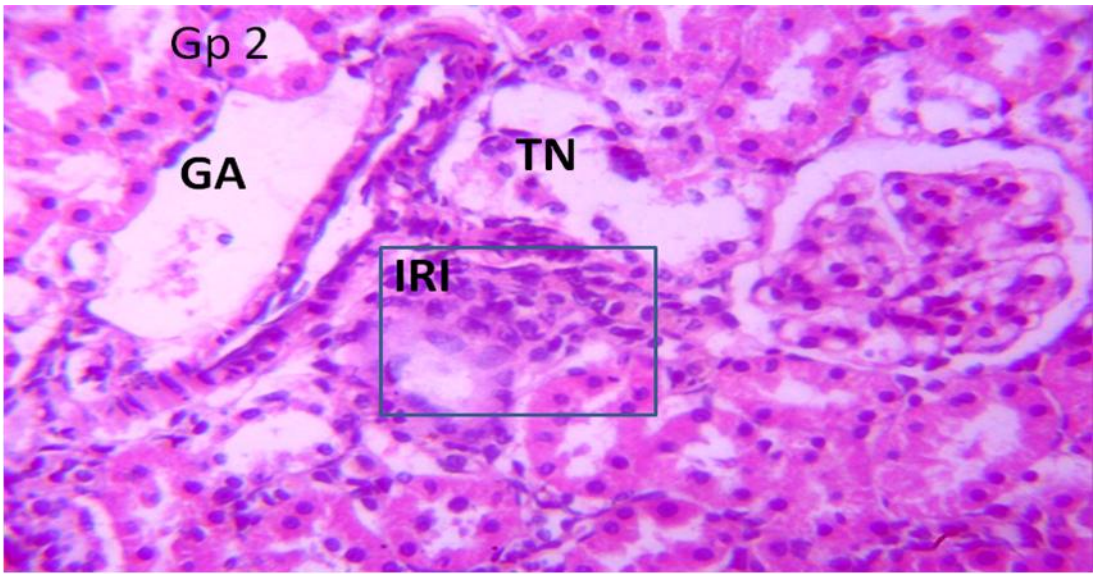

Figure 4.43 Photomicrograph of 2 section of kidney induced with anaemia show moderate to severe the renal tissue with severe aggregate of intra renal imflammation (IRI) , moderate glomerular attrophy (GA) and moderate tubular necrosis (TN).

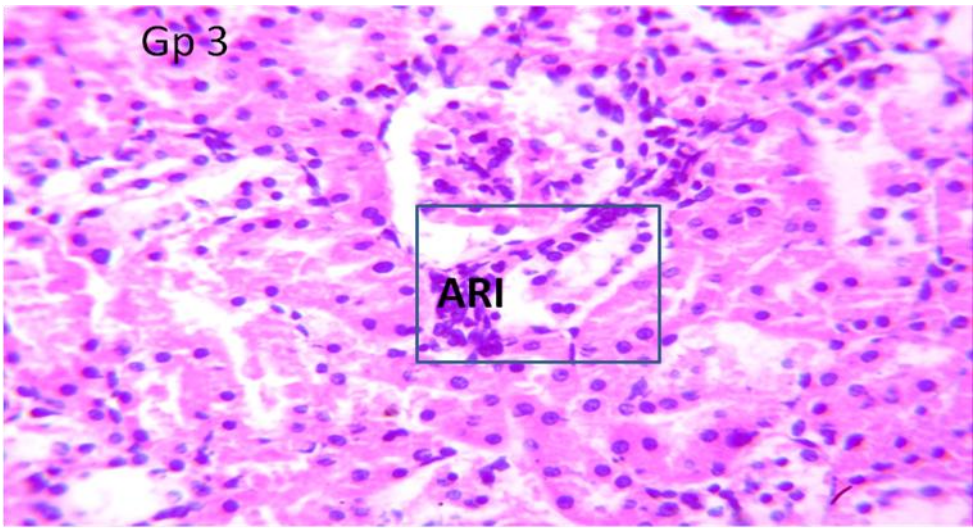

Figure 4.44 Photomicrograph of 3 section of kidney induced with anaemia and treated with standard drug (X400(H/E) show moderate healing with mild aggregate of renal inflammatory cell (ARI)arround the glomeruli.

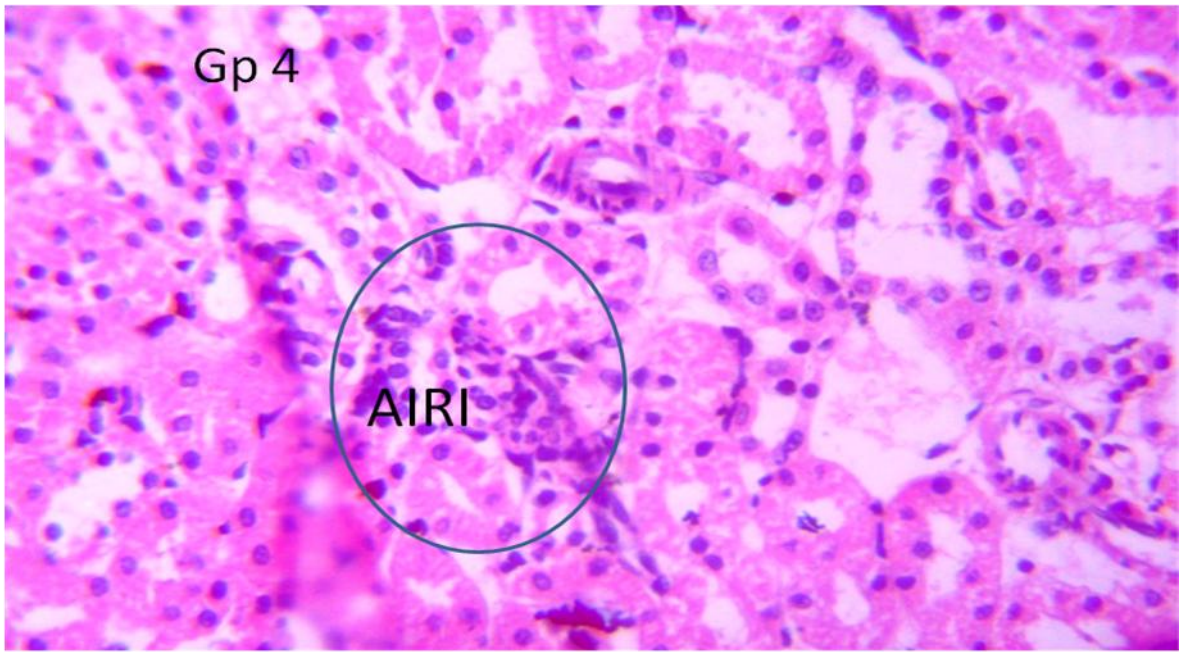

Figure 4.45 Photomicrograph of 4 section of kidney induced with anaemia and treated with 400mg/kg of MU1 (X400(H/E) show moderate healing with mild aggregate of intra renal inflammation (ARI)

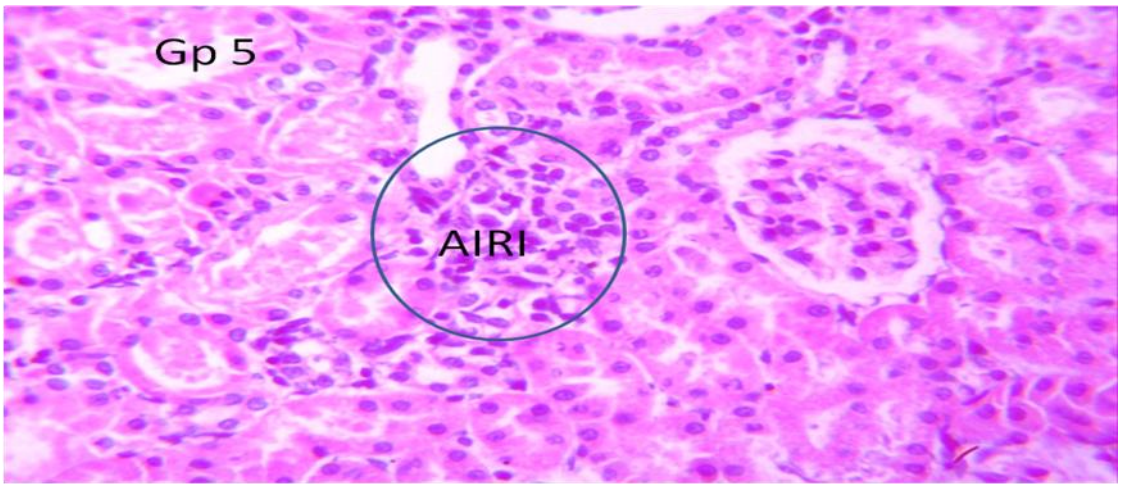

Figure 4.46 Photomicrograph of 5 section of kidney induced with anaemia and treated with 200mg/kg of MU1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI)

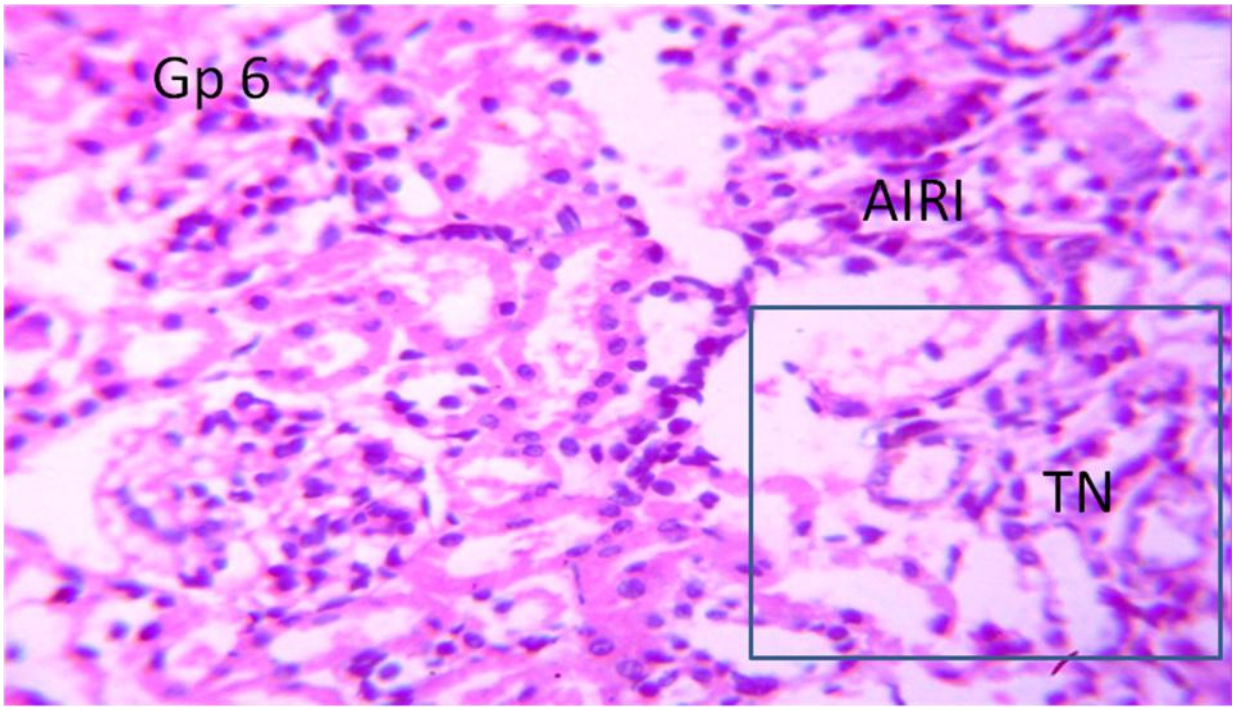

Figure 4.47 Photomicrograph of 6 section of kidney induced with anaemia and treated with 100mg/kg of MU1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) and moderate tubular necrosis (TN)

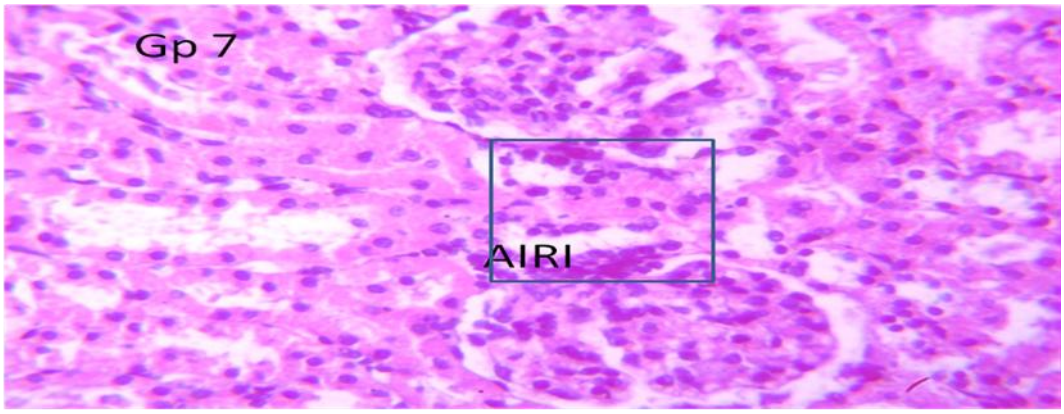

Figure 4.48 Photomicrograph of 7 section of kidney induced with anaemia and treated with 400mg/kg of TO1 (X400(H/E) show moderate healing with mild aggregate of intra renal inflammation (ARI) around the glomeruli .

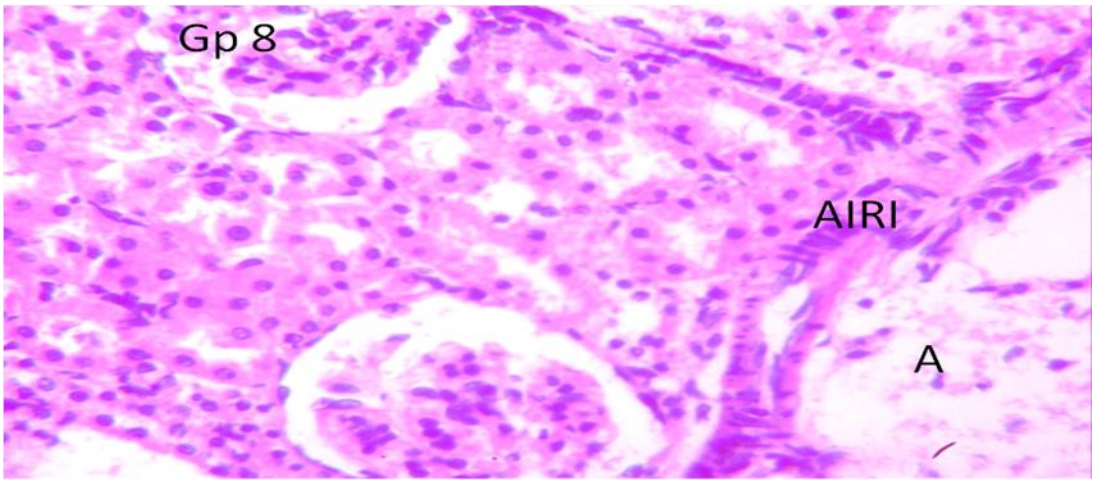

Figure 4.49 Photomicrograph of 8 section of kidney induced with anaemia and treated with 200mg/kg of TO1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) around the atrophied (A) glomeruli .

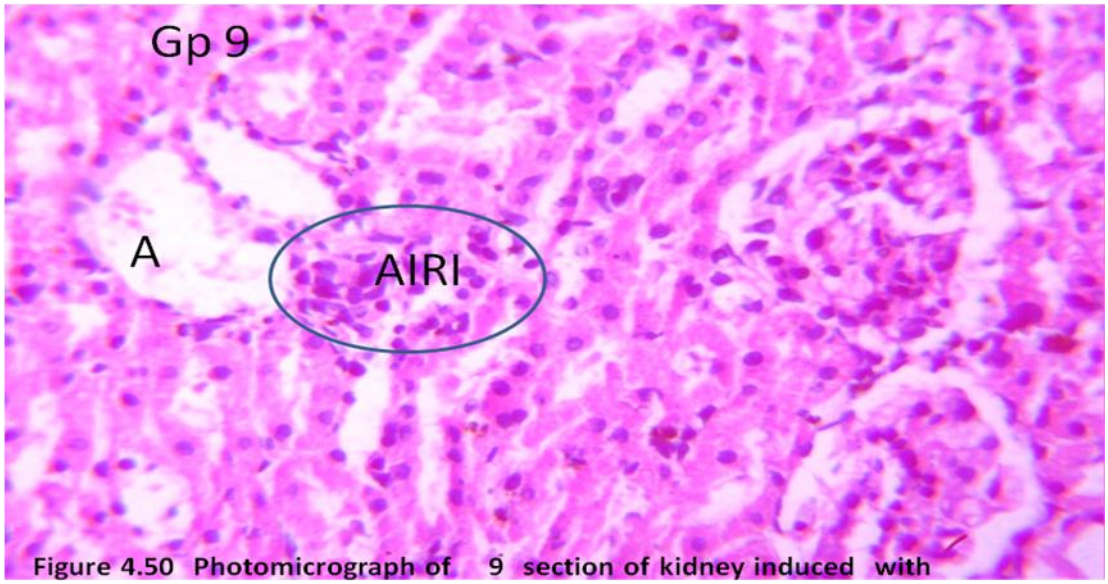

Figure 4.50 Photomicrograph of 9 section of kidney induced with anaemia and treated with 100mg/kg of TO1 (X400(H/E) show mild to moderate healing with mild aggregate of intra renal inflammation (ARI) around the atrophied (A) glomeruli .

DISCUSSION

The effect of ethanol leaf-extracts of *Mucuna poggei* (MU) and *Telfairia occidentalis* (TO) on kidney function parameters in phenylhydrazine-induced anaemia in Wister rats were also investigated. Kidneys are the primary route for haemoglobin clearance after saturation of the natural scavenging systems, and they are therefore highly susceptible to organ dysfunction during haemolysis. Renal lesions are described as major complications of haemolysis [28,29].Serum creatinine and blood urea have typically been used to diagnose kidney injury [30,31]. Higher levels of blood urea nitrogen and creatinine could be a sign of an underlying condition affecting the kidneys [32].The levels of

creatinine, blood urea and blood urea nitrogen (BUN) were found to be significantly elevated ($p < 0.05$) in rats that were treated with phenylhydrazine and induced with anaemia. However, administration of leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) and the standard multivitamin, HS-12 in phenylhydrazine -intoxicated rats substantially improved the phenylhydrazine -induced kidney damage as specified by suppressed levels of creatinine, blood urea and blood urea nitrogen (BUN) (figure 4.29 - 4.31). The observed results corroborate previous studies [33,34,35]. Phenylhydrazine is a non-immunogenic drug that generates changes in the red cell membrane, resulting in oxidative denaturation of hemoglobin (hb), and leading to the formation of an altered Hb called "Heinz bodies" which attenuates the life span of the erythrocytes [36]. Phenylhydrazine also causes numerous toxic and hazardous impacts, including nephrotoxicity, on human health. Its deposition in various organs causes oxidative stress that weakens the function of the native antioxidant system and, as a result, leads to the development of various severe pathological diseases [37].

Phenylhydrazine–induced renal damage may be associated with elevated serum levels of creatinine, blood urea and blood urea nitrogen (BUN) owing to leakage into the bloodstream [38]. The significant increase in urea and creatinine by phenylhydrazine administration observed in the present study suggested that the toxicant is nephrotoxic. Previous studies have equally observed an increase in creatinine and blood urea on exposure to dichlorvos toxicant [39]. Increase in haematological indices by the administration of leaf-extracts of *Mucuna poggei* (MU) and *Telfairia occidentalis* (TO) as observed in the present study (Table 4.10) suggested that the leaf-extracts of the two plants has the ability to stimulate erythropoietic factors capable of influencing the production of blood in the bone marrow. Erythropoietin is a maturation factor of RBC synthesis, which increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that is converted to RBCs and subsequently to mature erythrocytes [39,40]. It has been reported that kidney damage is associated with reduced erythropoietin production and excess breakdown of blood protein with a resultant decrease in hematological parameters [41]. Erythropoietin is a maturation factor of RBC synthesis, which increases the number of erythropoietin-sensitive committed stem cells in the bone marrow that is converted to RBCs and subsequently to mature erythrocytes [42]. More so, PHZ-induced erythrocyte deformity was suggested to decrease erythropoietin and corticosterone levels, and increase osmotic resistance and secondary tumour biomarkers to produce haematotoxicity in rodents. Recently, the effectiveness of recombinant human erythropoietin in an animal model of oxidative stress and genotoxicity was reported by [43]. The significant decrease in the haemoglobin concentrations may be due to either an increase in the rate at which the haemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis [44]. In addition to the established hemorrhage associated with phenylhydrazine toxicity [27] decreased haematological parameters observed in the present study may be partly attributed to impaired renal functions. Impaired renal function in phenylhydrazine-exposed rats, as evidenced by elevated serum urea and creatnine suggested the likelihood of kidney damage as the cause of decreased haematological parameters.

Photomicrograph of control section of kidney (X400)(H/E) showed well perfussed normal renal architecture with glomeruli (G) , bowman space (BS), renal tubules (RT)and tubular cell (TC) well outlined. Photomicrograph of kidney indused with anaemia showed moderate to severe renal tissue with severe aggregate of intra renal imflammation (IRI) ,moderate glomerular attrophy (GA) and moderate tubular necrosis (TN). Photomicrograph of kidney induced with anaemia and treated with standard drug (X400(H/E) showed moderate healing with mild aggregate of renal inflammatory cell (ARI)arround the

glomeruli (Figures 4.42 – 4.44). Photomicrograph of 4 section of kidney induced with anaemia and treated with 400mg/kg of MU1 (X400(H/E) showed moderate healing with mild aggregate of intra renal inflammation (ARI). Photomicrograph of kidney induced with anaemia and treated with 200mg/kg of MU1 (X400(H/E) showed mild healing with moderate aggregate of intra renal inflammation (ARI). Photomicrograph of kidney induced with anaemia and treated with 100mg/kg of MU1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) and moderate tubular necrosis (TN) (Figures 4. 4.45– 4.47). Photomicrograph of kidney induced with anaemia and treated with 400mg/kg of TO1 (X400(H/E) showed moderate healing with mild aggregate of intra renal inflammation (ARI) around the glomeruli. Photomicrograph of kidney induced with anaemia and treated with 200mg/kg of TO1 (X400(H/E) show mild healing with moderate aggregate of intra renal inflammation (ARI) around the atrophied (A) glomeruli . Photomicrograph of 9 section of kidney indused with Anaemia and treated with 100mg/kg of TO1 (X400(H/E) show mild to moderate healing with mild aggregate of intra renal inflammation (ARI) around the atrophied (A) glomeruli (Figures 4.48 – 4. 50). Major kidney damage was verified by the presence of significant damage to glomeruli in phenylhydrazine -treated rats. Figure: These findings corroborate those of Dardouri et al., (2016) who reported atrophy of glomerular capillaries and necrosis of proximal tubules. The findings of kidney function markers and oxidative stress markers—where leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) and standard multivitamin, HS-12 ameliorated phenylhydrazine -induced degenerative changes and improved kidney function owing to its possible antioxidant and anti-inflammatory effects—have been confirmed by histopathological observations. Our histological results on the PHZ-injected mice revealed minor but significant tubular dilatation, suggestive of tubular necrosis and were in tandem with the results of the kidney function parameters. These were in agreement with other models of haemolysis, including old blood transfusion in mice [7] and guinea pigs [9].

In our study, histological analysis revealed significant renal impairments in response to phenylhydrazine intoxication. These results are in agreement with those of previous studies [10]. In addition [30] reported that phenylhydrazine -induced glomeruli structural changes, increased the mesangial matrix, and swelling of the glomeruli with wider urinary spaces were detected. phenylhydrazine treatment resulted in tubular dysfunction and nuclear membrane damage in glomerular epithelial cells of rats [5]. However, Gobe and [8] attributed the relationship between phenylhydrazine -intoxication and renal cell injury to the sensitivity of the proximal tubular epithelium to oxidative stress. Similarly, [9] reported that increased nitric oxide and ROS generation was related to renal injury and induced the progression to renal failure. Leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) restored the normal architecture of the glomerulus and their antioxidant properties protected the kidneys of rats from nephrotoxicity [9]. Moreover, [10] suggested that catechin effectively protects renal tissue from nephrotoxicity, and propyl gallate in rats is therapeutic in response to diabetic glomerular endothelial proliferation [12].

Conclusion: The results of this study validates the antianaemic potentials of *Mucuna poggei* and, *Telfairia occidentalis* suggesting that the use of the leaves of these plants may potentiate a better cardioprotective, hepatoprotective, nephroprotective and increased antianaemic and antioxidant effects.

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