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Hepatoprotective and haemoprotective effects of ethanol leaf-extracts of *Mucuna poggei* and *Telfairia occidentalis* in phenyl-hydrazine-induced Liver damage in Wister albino rats.

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ABSTRACT

Several strategies for discovering drugs from unexplored natural products continue to strengthen research and development with current therapeutic evidence supporting their applications.The hepatoprotective and haemoprotective effects of ethanol leaf-extracts of *Mucuna poggei* and *Telfairia occidentalis* in phenyl-hydrazine-induced liver damage 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, RBC and Hb, significantly ($P<0.05$) decreased in anaemic control rats relative to the normal control rats, but were significantly ($P<0.05$) increased on treatment with the standard multivitamin HS-12 and the leaf-extracts. Serum AST, ALT and ALP and GGT activities were significantly ($P<0.05$) increased in the anaemic control group relative to the normal control and significantly ($P<0.05$) decreased on treatment with the leaf-extracts and the standard multivitamin. There was significant ($P<0.05$) increase in the levels of serum total bilirubin and conjugated bilirubin in the anaemic rats relative to the normal control. These however, decreased significantly ($P<0.05$) on treatment. There was significant ($P<0.05$) decrease in the levels of serum total protein in the anaemic rats relative to the normal control but this significantly ($P<0.05$) increased on treatment with the leaf-extracts and the standard multivitamin. Photomicrograph of the liver of the albino rats 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 that leaf-extracts of *Mucuna poggei* and *Telfairia occidentalis* has hepatoprotective and haemoprotective effects.

Keywords. *Telfairia occidentalis*, *Mucuna poggei,* phenylhydrazine and hepatoprotective

INTRODUCTION

The use of medicinal plants is a strategy by man to fight various diseases based on the fact that medicinal plants have nutritional and pharmacological biological activities of importance [1,2,3,4]. Herbs are useful in the search for new drugs because they are valuable sources of new molecules which may be scientifically modified to provide improved drugs [5,6]. Medicinal plants range from those used in the production of mainstream pharmaceutical products to plants used in herbal medicine. Plants that have medical uses can be found growing in many settings all over the world [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]. *Mucuna poggei* is one of such plants with physical characteristics that are repelling to both human and animals from getting closer. It is of the family: Fabaceae, Subfamily: Faboideae, Tribe: Phaseoleae, Subtribe: Erythrininae, Genus: Mucuna, Species: poggei and Variety: Mucuna poggei var. pesa [9,10]. In order to boost blood levels, most rural dwellers in some parts of Nigeria, especially the Igbo speaking people have resorted to oral administration of the crude aqueous extract of *Mucuna poggei* as the cheapest source of multivitamins. *Telfairia occidentalis* commonly known as "fluted pumpkin" is a seed and leave vegetable that is common in the West and Central Africa specifically in the forest zones, most commonly in Cameroon, Benin and Nigeria. The plant is well- known and a highly consumed vegetable all over Nigeria and is indigenous to southern Nigeria [11,12].

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 [13,14] 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 [15,16]. This condition of anaemia can also develop due to factors like pregnancy, malnutrition, blood parasite like helminthes infection trypanosome and plasmodium, long usage of some drugs like NSAIDs, long exposure to (poisonous) harmful chemicals, like phenylhydrazine. There are other causes of anaemia such as non-access to balance diet, folate and Vitamin B12 deficiencies, chronic inflammation and inherited disorders [17,18]. Anaemia occurs at all stages of the life cycle, but is more prevalent in pregnant women with increased risk of maternal and child mortality, lactating women, menstruating women and women of child bearing age [19,20]. Preschool children less than five years of age, adolescents, the elderly and people with chronic diseases are at increased risk of anaemia [21]. The liver is a vital organ that regulates a wide variety of biochemical processes and also plays an important role in the metabolism of carbohydrates, proteins and lipids [22]. Detoxification of potentially toxic chemicals, drugs and environmental contaminants is mainly related to the function of an applicable and healthy liver [23]. Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion of reduced glutathione levels. In addition, serum levels of many biochemical markers like transaminases, alkaline phosphatase, bilirubin, triglycerides and cholesterol are

elevated in liver disease. Liver diseases pose a serious challenge to international public health [24]. The factors progressing chronic liver diseases are free radical-induced oxidative stress and chronic inflammation resulting from the release of proinflammatory cytokines from the liver Kupffer cells [25]. The enzymatic and non-enzymatic defense systems of the normal hepatocytes protect them against free radicals and reactants; the enzymatic defense system includes catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) which rapidly react with and remove reactive oxygen species (ROSs) such as superoxide, hydrogen peroxide and hydroxyl radical [26]. The cell can tolerate mild oxidative stress; but in a more severe state, oxidative compounds or ROS react with cellular constituents such as DNA, lipids, proteins and cell membrane, and subsequently cause pathological problems [27]. Exposing the liver to a range of environmental toxic agents enhances hepatic injury [28]. A growing interest has emerged around the globe in rediscovering medicinal plants as useful therapeutic agents for the prevention of such injury [29,30]. Hepatotoxicity means dysfunction of the liver due to an overload of chemicals and drugs that are toxic to the body. Hepatotoxicity or liver injury, induced by drugs, is one of the major causes of liver diseases [31]. Phenylhydrazine is known to produce ROS, which results in oxidative stress. It is known that DNA, lipids and proteins are the main targets of oxidative injury [32,33,34,35]. Liver serves to filter out toxic substances from the bloodstream [36,37,38,39,40]. When there are excessive chemicals filtering through the liver, it becomes overloaded and can lead to hepatotoxicity [41,42,43,44].This current study investigated the hepatoprotective and hemoprotective effects of ethanol leaf-extracts of *Mucuna poggei* and *Telfairia occidentalis* in phenyl-hydrazine-induced liver damage in Wister albino rats.

MATERIALS AND METHODS

MATERIALS

Chemicals and Reagents

All chemicals and reagents used were of analytical grade.

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.

Extraction and Preparation of Leaf Sample

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.

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 [31]. 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. 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 vapour 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.

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

analyses served as markers for the antianaemic effects of the ethanol leaf-extracts of the two plants.

Determination of liver function Parameters

Activity of alanine aminotransferase (ALT) was assayed for by the method of Reitman and Frankel 1957 using Randox assay kits. Activity of Aspartate aminotransferase (AST) was assayed for by the method of Reitman and Frankel 1957 using Randox assay kits. The serum activity of alkaline phosphatase was quantified as described by Tietz (1995) using Randox assay kits. The activity of Gammaglutamyl transferase (GGT) was determined by the method of Szasz (1976). Total protein was determined using colorimetric Biuret method as described by Tietz (1995). The serum Total Bilirubin (TB) concentration was determined based on the method described by Tietz (1995) using Randox Kit (Randox laboratories limited UK). The determination of Serum Direct/Indirect Bilirubin (DB/IB) concentration was determined based on the method described by Tietz (1995) using the Randox Kit (Randox laboratories limited UK).

Histopathology

Histological and histochemical alterations in the liver were investigated as described by Jarrar and Taib (2012)**.** Histological sections were prepared from paraffin blocks and stained with haematoxylin and eosin (H and E) to examine changes in the morphology of the cells.

STATISTICAL ANALYSIS

The results were expressed as mean ± standard deviation (SD). The data were 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

Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on **PCV, RBC and HGB** counts of phenylhydrazine-induced anaemia in Wistar rats. The result on Table 1 showed the effect of ethanol leaf-extract of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on HCT (PCV) , Red Blood Cell (RBC) and Haemoglobin (HGB) count of phenylhydrazine-induced anaemia in Wistar rats. The results indicated that HCT (PCV), Red Blood Cell (RBC) and Haemoglobin (HGB) counts significantly ($P<0.05$) decreased in anaemic control rats relative to the normal control rats, but were significantly ($P<0.05$) increased on treatment with the standard multivitamin and ethanol leaf-extract of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) at various concentrations of 100, 200 and 400 mg/kg body weight.

Table 1: Antianaemic effect of leaf-extracts of *M poggei* and *T. occidentalis* and HS12 on PCV, RBC and Hb in the different experimental groups.

Groups (n=6)	HCT (PCV) (%)	RBC ($\times 10^{12} ul^{-1}$)	HB(g/dL)
NC	38.5±0.84	6.59±0.49	12.55±1.34
AC	25.8±1.13^{*,a}	5.6±0.40	12.30±0.85
PHZ+ HS-12 (SC) (0.1ml)	45.6±3.96	6.14±0.01	13.95±0.35
PHZ+ 400mg/Kg (MP1)	45.85±2.33	6.26±0.26	14.15±0.35
PHZ+ 200mg/Kg (MP2)	34.0±8.91^{c,d}	5.04±1.53	11.50±2.69
PHZ+ 100mg/Kg (MP3)	48.10±0.71	6.95±0.46	14.85±0.64
PHZ+ 400mg/Kg (TO1)	43.80±0.71	6.55±0.49	13.60±0.71
PHZ+ 200mg/Kg (TO2)	43.15±2.33	6.67±0.50	13.00±0.28
PHZ+ 100mg/Kg (TO3)	30.55±3.46^{e,f}	3.68±0.70	9.60±2.69

Values are expressed as Mean ± SD ($p<0.05$) *Significantly different vs NC, [a]Significantly different vs other groups, [c]Significantly different vs MP1, [d]Significantly different vs MP_2, [e]Significantly different vs TO_2, [f]Significantly different vs TO_1.
PCV: Packed Cell Volume, RBC: Red Blood Cells, Hb: Haemoglobin.

Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Alanine Amino Transferase (ALT) activity of phenylhydrazine-induced anaemia in Wistar rats.

Serum ALT activity increased significantly (P<0.05) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly (P<0.05) decreased the activity of this liver function parameter (Figure 1). The results showed that the *Telfairia occidentalis* (TO) leaf-extracts induced a significantly (P<0.05) lower serum ALT activity at the 400 mg/Kg (TO1) and 200 mg/Kg body weight (TO2) concentrations relative to the leaf-extracts of *Mucuna poggei* (MP). However, these decreases in serum ALT activity of the leaf-extracts of *Mucuna poggei* (MP) the 400 mg/Kg (MP1) and 200 mg/Kg body weight (MP2) were similar to that of the standard control (SC) but lower than that of the normal control (NC). The results also showed that there was no significant difference between the serum ALT

activity of the leaf-extracts of *Mucuna poggei* (MP3) and *Telfairia occidentalis* at 100 mg/Kg body weight (TO3) levels of treatments (Figure 1).

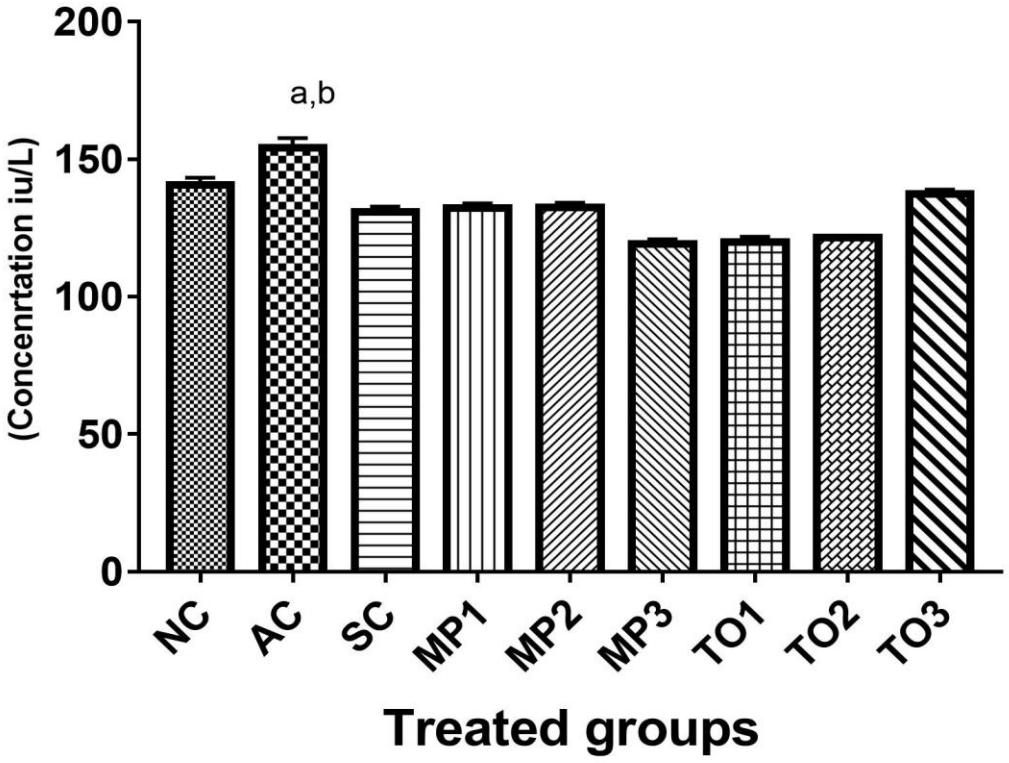


Figure 1. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Alanine Amino Transferase (ALT) activity of phenylhydrazine-induced anaemia in Wistar rats.[a] Significantly different vs NC, [b] Significantly different vs all treatment groups. n=5 mean ± SD *($p < 0.05$).* NC- Normal control, AC- Anemic 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 Aspartate Amino Transferase (AST) activity of phenylhydrazine-induced anaemia in Wistar rats.

Serum AST activity increased significantly ($P<0.05$) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly ($P<0.05$) decreased the activity of this liver function parameter (Figure 2). The results showed that the *Telfairia occidentalis* (TO) leaf-extracts induced a significantly ($P<0.05$) lower serum AST activity at the 400 mg/Kg (TO1) and 200 mg/Kg body weight (TO2) concentrations relative to the leaf-extracts of *Mucuna poggei* (MP). However, these decreases in serum AST activity of the leaf-extracts of *Telfairia occidentalis* (TO) at the 400 mg/Kg (MP1) and 200 mg/Kg body weight (MP2) were similar to that of the standard control (SC) but lower than that of the normal control (NC) (Figure 2). The results also showed that serum AST activity increased significantly ($P<0.05$) at the 100 mg/Kg body weight (TO3) of the leaf-extracts of *Telfairia occidentalis* than that of *Mucuna poggei* (MP3) (Figure 2).

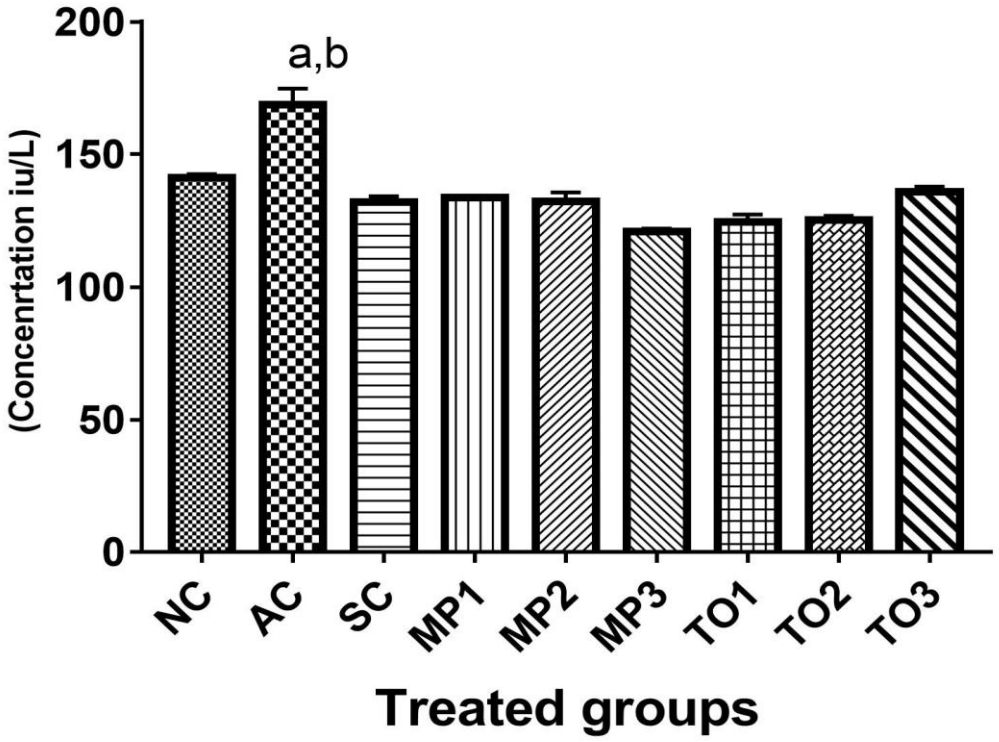


Figure 2. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Aspartate Amino Transferase (AST) activity of phenylhydrazine-induced anaemia in Wistar rats.[a] Significantly different vs NC, [b] Significantly different vs all treatment groups. n=5 mean ± SD *($p < 0.05$).* NC- Normal control, AC- Anaemic control, SC- Standard control, MP1, 2 and 3. *M. poggei* at varying concentrations, TO1, 2 and 3. *T. occidentals* at different concentrations.

Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Aspartate Amino Transferase (AST) activity / Alanine Amino Transferase (ALT) activity ratio (AST / ALT) of phenylhydrazine-induced anaemia in Wistar rats.

Serum Aspartate Amino Transferase (AST) activity / Alanine Amino Transferase (ALT) activity ratio (AST / ALT) increased significantly (P<0.05) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly (P<0.05) decreased this ratio (Figure 3). The results showed that the serum Aspartate Amino Transferase (AST) activity / Alanine Amino Transferase (ALT) activity ratio (AST / ALT) of *Telfairia occidentalis* (TO) leaf-extracts induced similar serum AST activity at the 400 mg/Kg (TO1), 200 and mg/Kg (TO2) and 100 mg/Kg body weight (TO3) concentrations relative to the leaf-extracts of *Mucuna poggei* (MP). However, these similar serum (AST / ALT) activity ratios of the leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) were similar to that of the standard control (SC) but lower but not significantly (P>0.05) than that of the normal control (NC) (Figure 3).

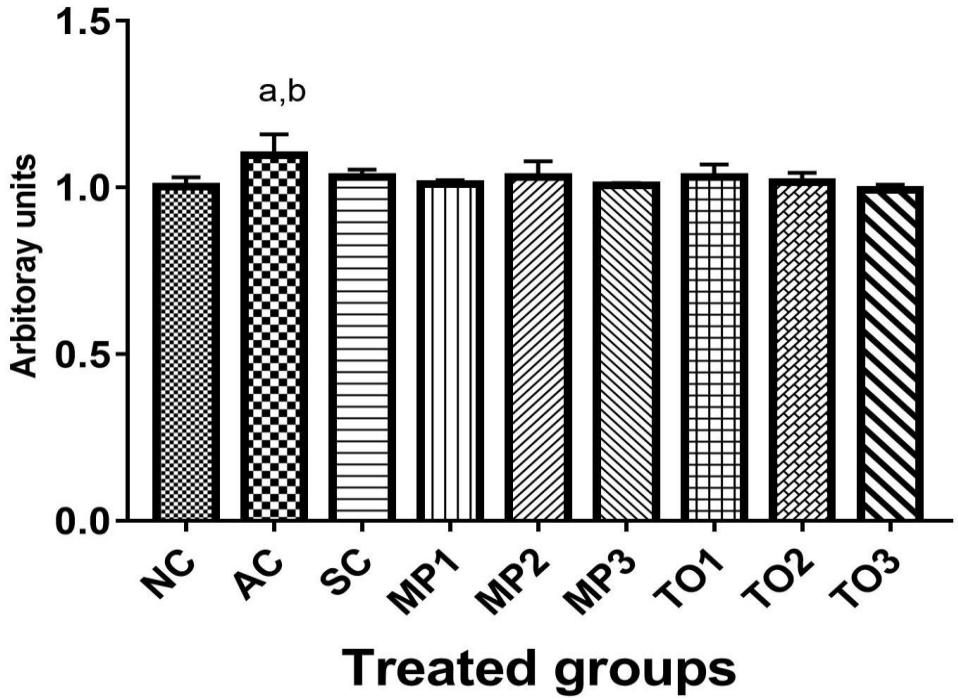


Figure 3. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Aspartate Amino Transferase (AST) activity / Alaline Amino Transferase (ALT) activity ratio (AST / ALT) of phenylhydrazine-induced anaemia in Wistar rats. [a] Significantly different vs NC, [b] Significantly different vs all treatment groups. n=5 mean ± SD *($p < 0.05$).* 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 Alkaline Phosphatase (ALP) activity of phenylhydrazine-induced anaemia in Wistar rats.

Serum ALP activity increased significantly (P<0.05) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly (P<0.05) decreased the activity of this liver function parameter (Figure 4). The results showed that the leaf-extracts of *Mucuna poggei* (MP) induced a significantly (P<0.05) lower serum ALP activity at the 400 mg/Kg (MP1) and 200 mg/Kg body weight (MP2) concentrations relative to the leaf-extracts of *Telfairia occidentalis* (TO). However, these decreases in serum ALP activity of the leaf-extracts of *Mucuna poggei* (MP) at the 400 mg/Kg (MP1) and 200 mg/Kg body weight (MP2) were similar to that of the standard control (SC) and the normal control (NC) (Figure 4). The results also showed that serum ALP activity decreased significantly (P<0.05) at the 100 mg/Kg body weight of *Mucuna poggei* (MP3) than that of the leaf-extracts of *Telfairia occidentalis* (TO3). However, this decrease in serum ALP activity of the leaf-extracts of *Mucuna poggei* (MP) at the 100 mg/Kg body weight (MP3) was significantly (P<0.05) lower than that of the standard control (SC) and the normal control (NC) (Figure 4).

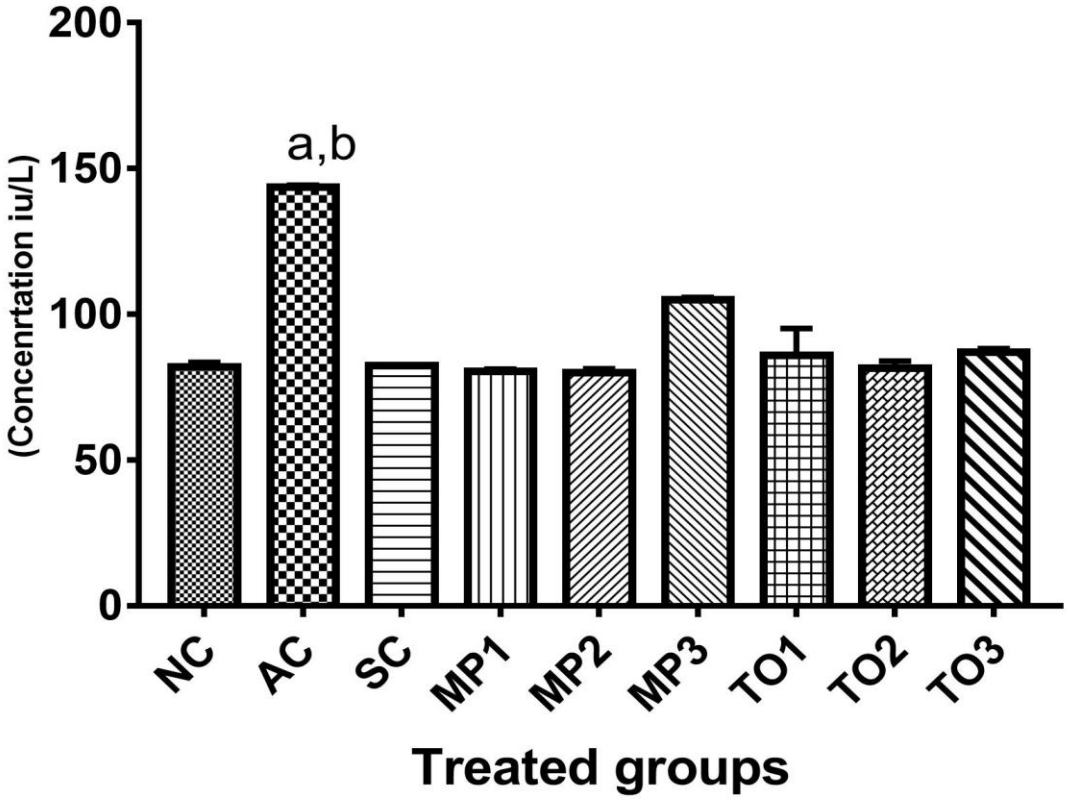


Figure 4. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Alkaline Phosphatase (ALP) activity of phenylhydrazine-induced anaemia in Wistar rats. [a] Significantly different vs NC, [b] Significantly different vs all treatment groups. n=5 mean ± SD *($p < 0.05$).* 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 Gamma Glutamyl Transferase (GGT) activity of phenylhydrazine-induced anaemia in Wistar rats.

Serum GGT activity increased significantly (P<0.05) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly (P<0.05) decreased the activity of this liver function parameter (Figure 5). The results showed that the *Telfairia occidentalis* (TO) leaf-extracts induced a significantly (P<0.05) lower serum GGT activity at the 400 mg/Kg (TO1) and 200 mg/Kg body weight (TO2) concentrations relative to the leaf-extracts of *Mucuna poggei* (MP). However, these decreases in serum GGT activity of the leaf-extracts of *Telfairia occidentalis* (TO) at the 400 mg/Kg (MP1) and 200 mg/Kg body weight (MP2) were significantly (P<0.05) lower than that of the standard control (SC) but lower than that of the normal control (NC) and significantly (P<0.05) higher than that of the normal control (NC) (Figure 5). The results also showed that serum GGT activity decreased significantly (P<0.05) at the 100 mg/Kg body weight of the leaf-extracts of *Mucuna poggei* (MP3) than that *Telfairia occidentalis* (TO3). However, this decrease in serum GGT activity of the leaf-extracts of *Mucuna poggei* (MP) at the 100 mg/Kg body weight (MP3) was significantly (P<0.05) lower

than that of the standard control (SC) and significantly (P<0.05) higher than that of the normal control (NC) (Figure 5).

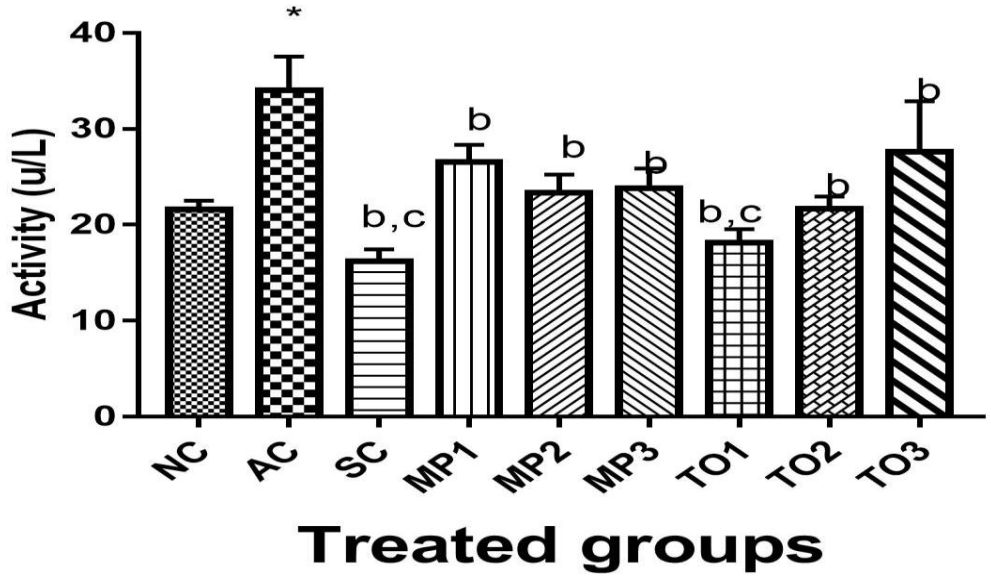


Figure 5 Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Gamma Glutamyl Transferase (GGT) activity of phenylhydrazine-induced anaemia in Wistar rats. [*]Significantly different vs NC, [b] significantly different vs AC, [c] Significantly different vs all other groups n=5 mean ± SD *(p < 0.05).* . 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 Total Protein (TP) concentration of phenylhydrazine-induced anaemia in Wistar rats.

There was significant (P<0.05) decrease in the levels of serum total protein in the anaemic rats relative to the normal control. These were significantly (P<0.05) increased on treatment with the leaf-extracts and the standard multivitamin (Figure 6). The results showed that *Telfairia occidentalis* (TO) leaf-extracts induced a significantly (P<0.05) higher levels of serum total protein at the 400 mg/Kg (TO1) relative to the leaf-extracts of *Mucuna poggei* (MP1) which was similar to the normal control (NC) but higher than the standard control (SC). Conversely, *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) higher levels of serum total protein at the 200 mg/Kg (MP2) relative to the leaf-extracts of *Telfairia occidentalis* (TO2) which was significantly (P<0.05) lower than both the normal control (NC) and the standard control (SC) (Figure 6). The results also showed that serum total protein decreased significantly (P<0.05) at the 100 mg/Kg body weight of the leaf-extracts of *Mucuna poggei* (MP3) than that of *Telfairia occidentalis* (TO3). However, this decrease in serum total protein of the leaf-extracts of *Mucuna poggei* (MP) at the 100 mg/Kg body weight (MP3) was significantly (P<0.05) lower than that of the standard control (SC) and the normal control (NC) (Figure 6).

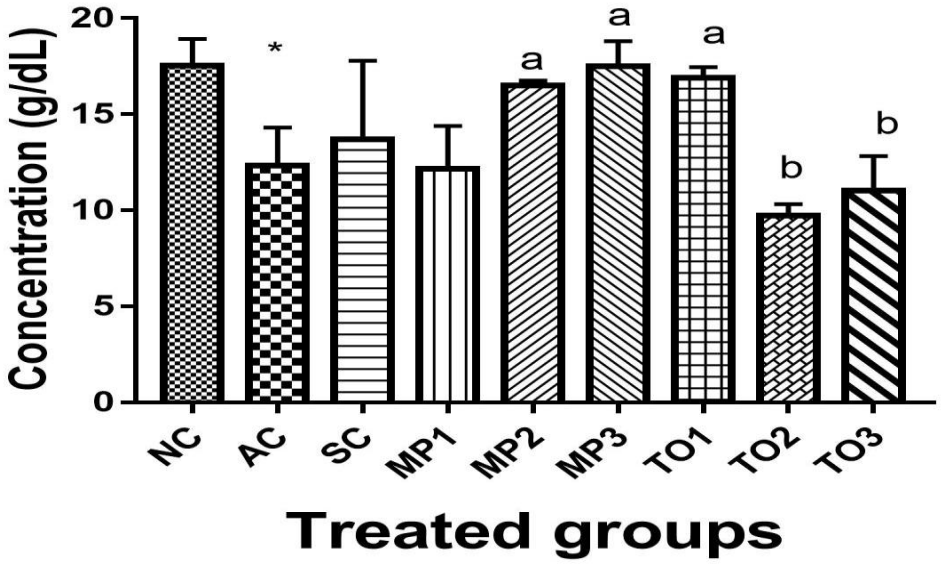


Figure 6. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Total Protein (TP) concentration of phenylhydrazine-induced anaemia in Wistar rats. *Significantly different vs NC, [a] significantly different vs AC, [b]Significantly different vs all other groups 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.

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

There was significant (P<0.05) increase in the levels of serum total bilirubin in the anaemic rats relative to the normal control. Treatment with the crude ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* at 100, 200 and 400 mg/kg body weight and the standard multivitamin significantly (P<0.05) reduced the serum levels of these parameters in a dose dependent manner (Figure 7). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) lower level of serum total bilirubin at the 400 mg/Kg (MP1) relative to the leaf-extracts of *Telfairia occidentalis* (TO1) which was significantly (P<0.05) lower than the normal control (NC) and the standard control (SC). Conversely, *Telfairia occidentalis* (TO) leaf-extracts induced a significantly (P<0.05) lower levels of serum total bilirubin at the 200 mg/Kg (TO2) relative to the leaf-extracts of *Mucuna poggei* (MP) which was significantly (P<0.05) lower than both the normal control (NC) and the standard control (SC) (Figure 7). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) lower level of serum total bilirubin at the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO3) which was significantly (P<0.05) lower than the normal control (NC) and the standard control (SC) (Figure 7).

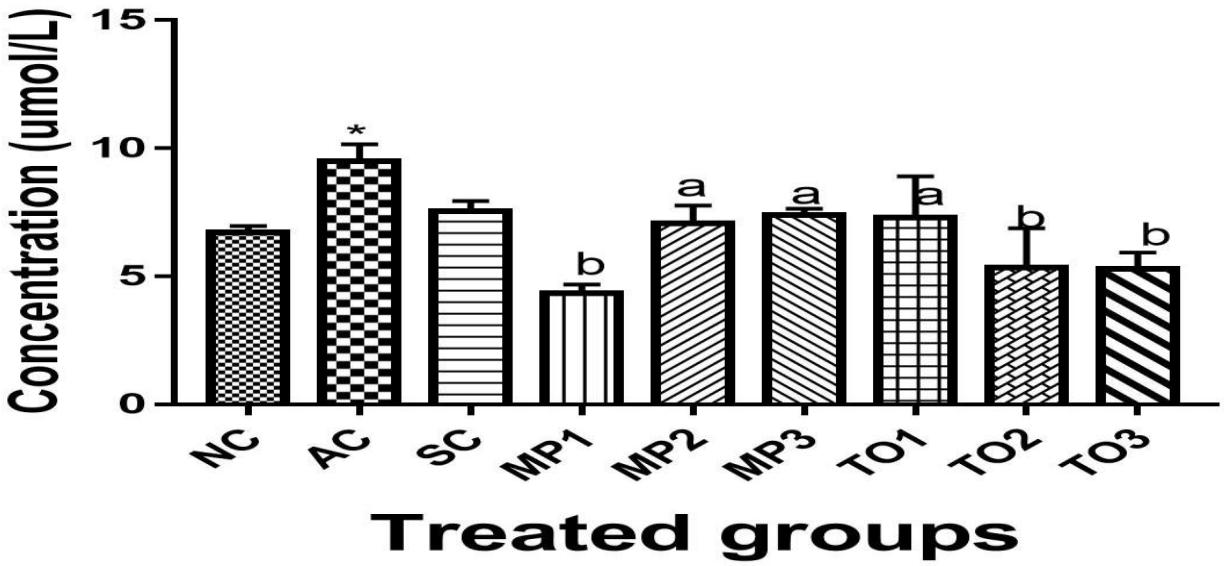


Figure 7. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Total Bilirubin (TB) concentration of phenylhydrazine-induced anaemia in Wistar rats. *Significantly different vs NC, [a] significantly different vs AC, [b]Significantly different vs all other groups n=5 mean ± SD *(p < 0.05).* NC-Normal control, AC- Anaemic control, SC-Standard control, MP1, 2 and 3. *M. poggei* at varying concentrations, TO 1, 2 and 3. *T. occidentalis* at different concentrations.

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

There was significant (P<0.05) increase in the levels of serum direct / conjugated bilirubin in the anaemic rats relative to the normal control. Treatment with the crude ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* at 100, 200 and 400mg/kg body weight and the standard multivitamin significantly (P<0.05) reduced the serum levels of these parameters in a dose dependent manner (Figure 8). The results showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) lower level of serum direct / conjugated bilirubin at the 400 mg/Kg (MP1) relative to the leaf-extracts of *Telfairia occidentalis* (TO1) which was similar to the normal control (NC) but significantly (P<0.05) lower than the standard control (SC). Conversely, *Telfairia occidentalis* (TO) leaf-extracts induced a significantly (P<0.05) lower level of serum direct / conjugated bilirubin at the 200 mg/Kg (TO2) relative to the leaf-extracts of *Mucuna poggei* (MP) which was similar to the normal control (NC) but significantly (P<0.05) lower than the standard control (SC). (Figure 8). The results also showed that *Mucuna poggei* (MP) leaf-extracts induced a significantly (P<0.05) higher level of serum direct / conjugated bilirubin at the 100 mg/Kg (MP3) relative to the leaf-extracts of *Telfairia occidentalis* (TO3) which was significantly (P<0.05) lower than the normal standard control (SC) but similar to the normal control (SC) (Figure 8).

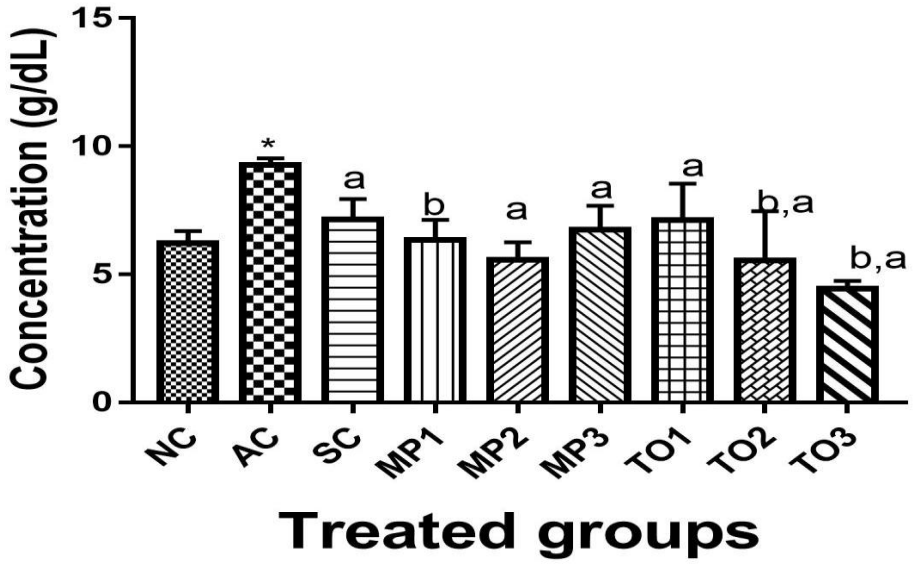
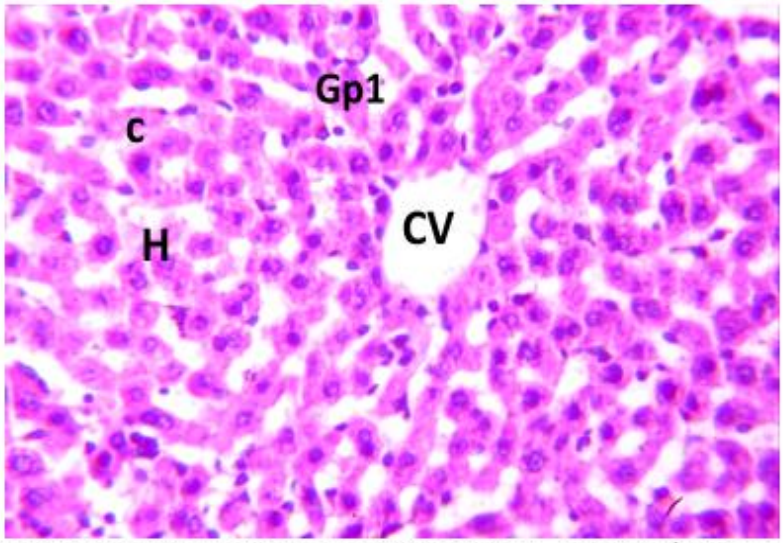


Figure 8. Effect of ethanol leaf-extracts of *Mucuna poggei* (MP) and *Telfairia occidentalis* (TO) on serum Direct/ conjugated Bilirubin concentration of phenylhydrazine-induced anaemia in Wistar rats. *Significantly different vs NC, [a] significantly different vs AC, [b]Significantly different vs all other groups n=5 mean ± SD *(p < 0.05).* 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.

Histopathology of theLiver

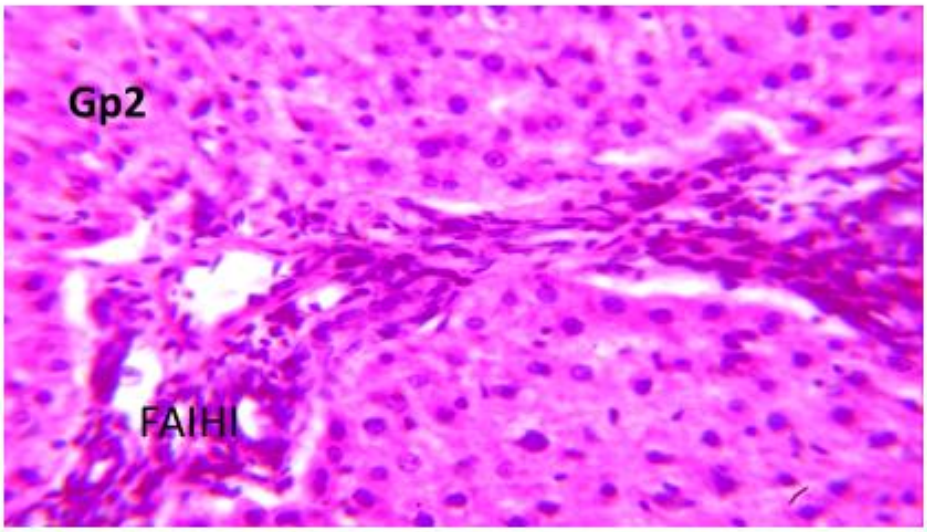
Photomicrograph of group 1 control section of liver (x400) (H/E) shows normal hepatic architecture with central vain (CV) hepatocyte(H) and well perfussed cytoplasm (C). Photomicrograph of group 2 section of liver induced with Anaemia (x400) (H/E) shows sever degeneration with sever focal aggregate of intra hepatic inflammation (FAIHI). Photomicrograph of group 3 section of liver induced with Anaemia and treated with 400mg/kg of MU1 (x400) (H/E) shows moderate healing with mild focal aggregate of intra hepatic inflammation (FAIHI). Photomicrograph of group 4 section of liver induced with Anaemia and treated with 200mg/kg of MU1 (x400) (H/E) shows moderate healing with mild focal aggregate of intra hepatic inflammation (FAIHI). Photomicrograph of group 5 section of liver induced with Anaemia and treated with 200mg/kg of MU1 (x400) (H/E) shows mild healing with moderate portal aggregate of inflammatory cell (PAIC). Photomicrograph of group 6 section of liver induced with Anaemia and treated with 100mg/kg of MU1 (x400) (H/E) shows mild healing with moderate intra hepatic inflammation(IHI) and cytoplasmic ground glass appearance(CGGA). Photomicrograph of group 7 section of liver induced with Anaemia and treated with 400mg/kg of TO1 (x400) (H/E) shows moderate healing with mild aggregate of intra hepatic inflammation(IHI). Photomicrograph of group 8 section of liver induced with Anaemia and treated with 200mg/kg of TO1 (x400) (H/E) shows mild healing with moderate congestion of the central vain (CCV)and moderate aggregate of inflammatory cell (AIC) arround the congested vain. Photomicrograph of group 9 section of liver induced with Anaemia and treated with 100mg/kg of TO1 (x400) (H/E) shows moderate healing with mild portal aggregate of inflammatory cell (PAIC) . Generally, photomicrograph of the liver 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 results of the biochemical parameters (Figures 9-17).



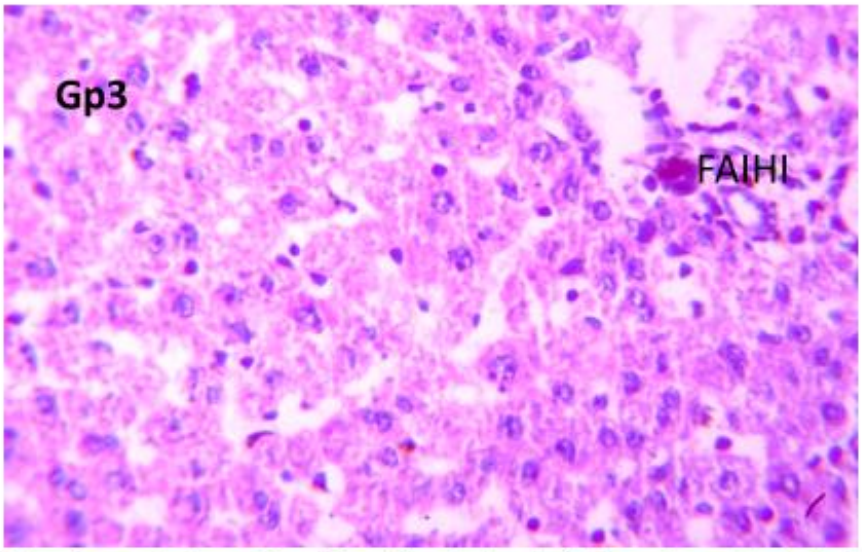
Photomicrograph of group 1 control section of liver (x400) (H/E) shows normal hepatic architecture with central vain (CV) hepatocyte(H) and well perfussed cytoplasm (C)

Figure 9



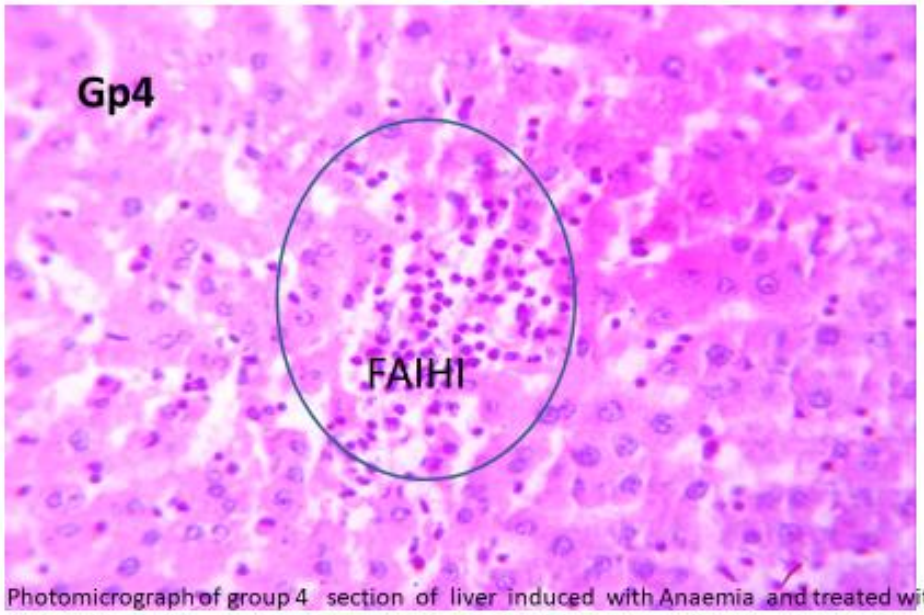
Photomicrograph of group 2 section of liver induced with Anaemia (x400) (H/E) shows sever degeneration with severe focal aggregate of intra hepatic inflammation (FAIHI).

Figure 10



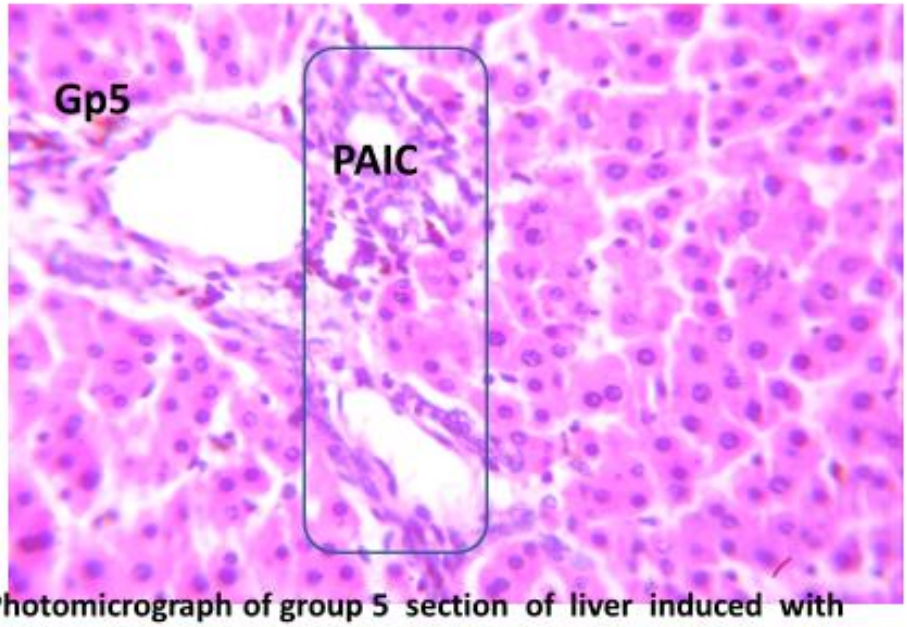
Photomicrograph of group 3 section of liver induced with Anaemia and treated with 400mg/kg of MU1 (x400) (H/E) shows moderate healing with mild focal aggregate of intra hepatic inflammation (FAIHI).

Figure 11



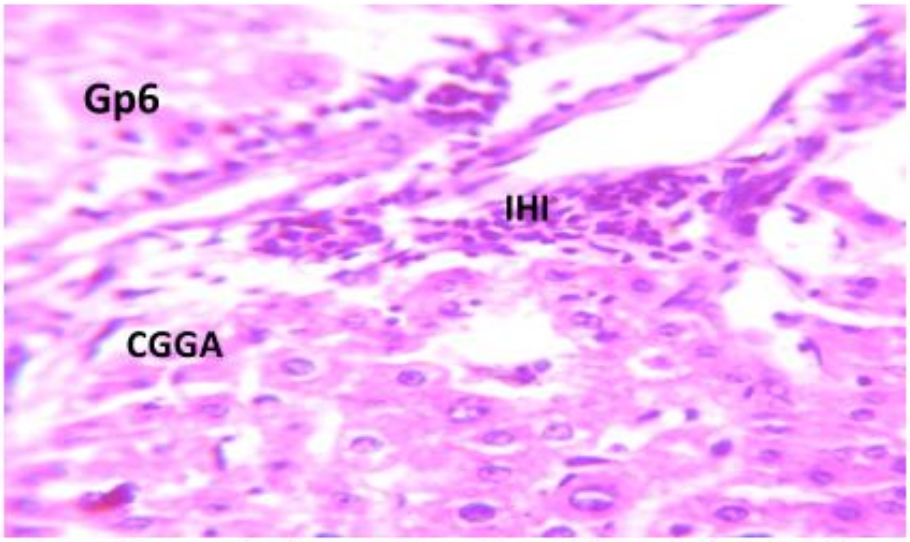
Photomicrograph of group 4 section of liver induced with Anaemia and treated with 200mg/kg of MU1 (x400) (H/E) shows moderate healing with mild focal aggregate of intra hepatic inflammation (FAIHI).

Figure 12



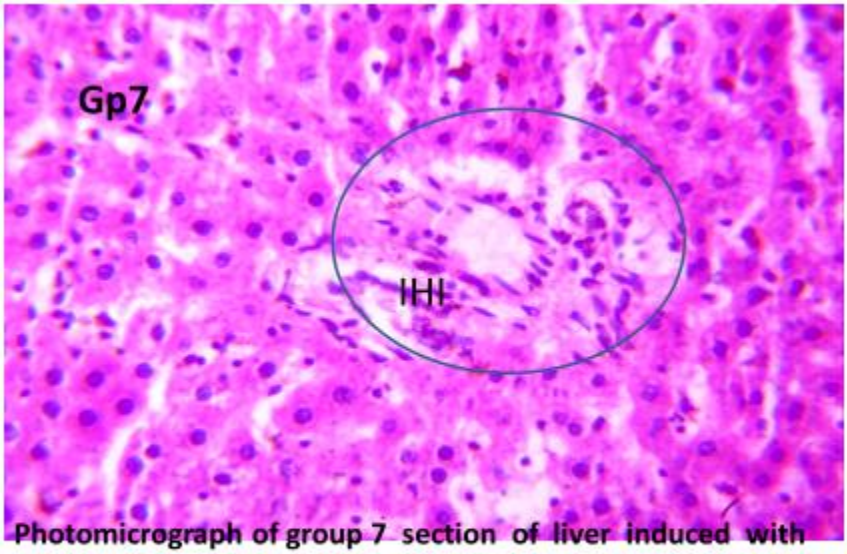
Photomicrograph of group 5 section of liver induced with Anaemia and treated with 200mg/kg of MU1 (x400) (H/E) shows mild healing with moderate portal aggregate of inflammatory cell (PAIC) .

Figure 13



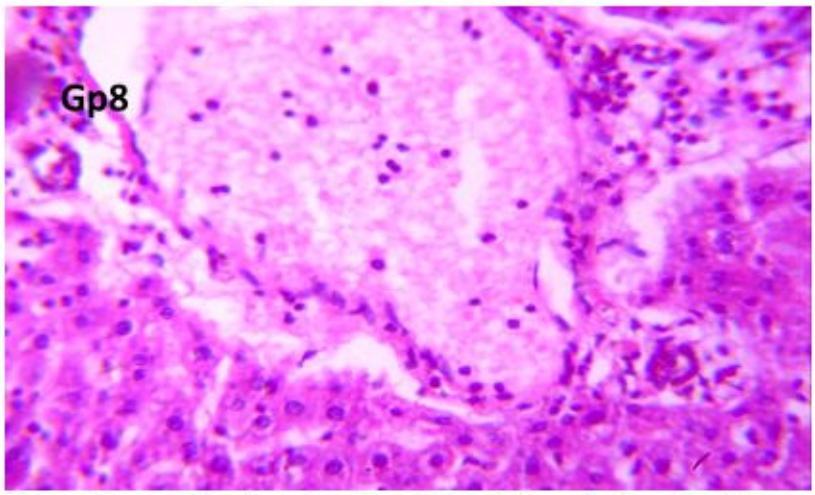
Photomicrograph of group 6 section of liver induced with Anaemia and treated with 100mg/kg of MU1 (x400) (H/E) shows mild healing with moderate intra hepatic inflammation(IHI) and cytoplasmic ground glass appearance(CGGA)

Figure 14



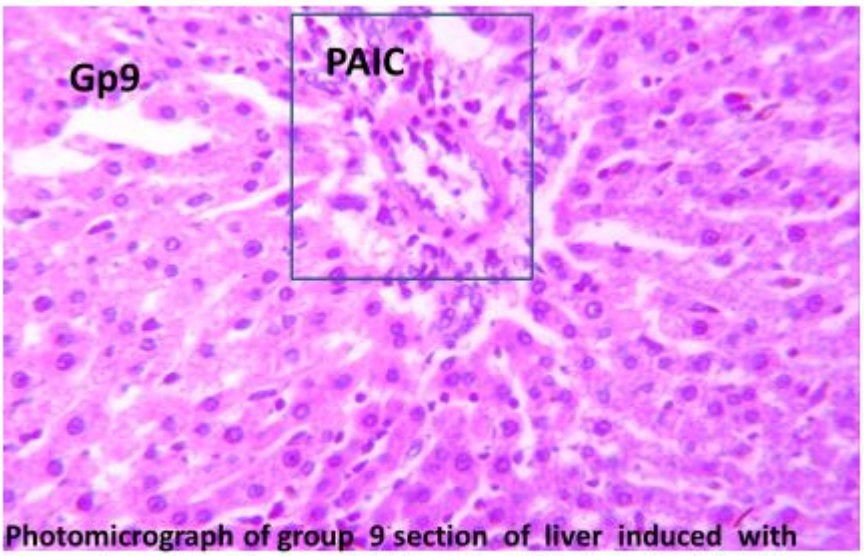
Photomicrograph of group 7 section of liver induced with Anaemia and treated with 400mg/kg of TO1 (x400) (H/E) shows moderate healing with mild aggregate of intra hepatic inflammation(IHI) .

Figure 15



Photomicrograph of group 8 section of liver induced with Anaemia and treated with 200mg/kg of TO1 (x400) (H/E) shows mild healing with moderate congestion of the central vain (CCV)and moderate aggregate of inflammatory cell (AIC) arround the congested vain .

Figure 16



Photomicrograph of group 9 section of liver induced with Anaemia and treated with 100mg/kg of TO1 (x400) (H/E) shows moderate healing with mild portal aggregate of inflammatory cell (PAIC)

Figure 17

DISCUSSION

The liver is the organ most commonly involved in the metabolism of endogenous and foreign compounds. Blood is transported to the liver through the portal vein which carries blood containing digested nutrients from the gastrointestinal tract and the hepatic artery which carries oxygenated blood from the lungs [45,46].The liver serves to filter out toxic substances from the bloodstream [8]. The Liver is the center of drug metabolism, when there are excessive chemicals filtering through the liver, it becomes overloaded and can lead to hepatotoxicity, also in the case of an overdose, the liver is overwhelmed by the drug and this can lead to oxidative stress and toxicity [11]. Hepatotoxicity means dysfunction of the liver due to an overload of chemicals and drugs that are toxic to the body. PH is known to produce ROS, which results in oxidative stress. It is known that DNA, lipids and proteins are the main targets of oxidative injury [12]. Liver function tests help in the diagnosis of any abnormal/normal condition of liver. Leakage of cellular enzymes into serum indicates the sign of hepatic tissue damage [17]. In hepatotoxicity, the transport function of liver cells is altered, causing an alteration of the plasma membrane, which results in an outflow of these liver enzymes that leads to an increase in their serum level [9]. Liver function parameters used to detect the presence of liver disease or potential harm to the liver in the present study were serum level of the enzymes AST, ALT and Alkaline phosphatase, GGT, total protein, total and conjugated / direct Bilirubin levels. In the present study, the PHZ-induced hepatotoxicity model was developed to examine the antioxidant and hepatoprotective effects of ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* on hepatic injury. The hepatotoxicity induced by PHZ is a reliable model for the study of hepatoprotective factors, which has been used in several studies in various intraperitoneal and oral doses for hepatotoxicity induction [20,26].

Aminotransferases are the most frequently used and most specific indicators of hepatic injury and represent markers of hepatocellular necrosis [9]. Liver enzymes AST and ALT are frequently used as biomarkers of liver

injury because they are released by hepatocytes into the extracellular space [18]. Usually, any kind of liver injury can cause a rise in ALT [15] and the release of ALT and AST from the cytosol occurs when there is injury to hepatocytes, especially in membrane damage [18]. The ALT and AST are transaminase enzymes that play a crucial role in converting amino acids into keto acids. The AST is predominantly present in the mitochondria of hepatocytes, and the ALT is mainly in hepatocyte cytoplasm; an increase in the ALT level is usually associated with an increase in the AST level [9,10]; however, the ALT is more specific for liver tissue, and therefore, a better marker for liver damage [11]. As it was observed in the current study, the ALT and AST levels were increased markedly in the anaemic group rats compared with that in the normal rats. Phenylhydrazine damages to hepatocytes, reduces cell membrane integrity, and consequently increases cellular leakage of transaminase enzymes [17]. Usually, any kind of liver injury can cause a rise in ALT and AST [20] and the release of ALT and AST from the cytosol occurs when there is injury to hepatocytes, especially in membrane damage [14]. In this present study, increased activities of marker enzymes were seen in the serum and were signs of cellular damage due to administration of phenylhyrazine. Serum AST and ALT activities increased significantly ($P<0.05$) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly ($P<0.05$) decreased the activities of these liver function parameters (Figure1-2). Increase in value of AST with damaging effects of hepatocellular injury led to AST leakage in circulating blood, which is in line with a previous study [7,9]. The significant ($P < 0.05$) increase in AST and ALT recorded in the anaemic control group by this study (Figure 1-2) indicated that phenylhydrazine is hepatoxic [15]. This finding is similar to another report [11,16] in which phenylhyrazine induction increased serum levels of AST and ALT . Many other chemicals have been documented to cause hepatotoxicity with the concomitant increase in serum levels of liver function parameters such as AST, ALT, ALP, they include acetaminophen (APAP), N'-Nitrosodimethylamine and lead[15,20,27]. Other examples are reports by [27,29] in which the hepatotoxic chemical bisphenol A increased serum AST, ALT, ALP . Also Cadmium has been implicated in hepatotoxicity as reported by [30].

Treatment with ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* at 100, 200 and 400 mg/kg body weight and the standard multivitamin significantly ($P < 0.05$) reduced ALT and AST activities to almost normal levels when compared to phenylhydrazine-treated group (Figure 1-2). This indicated liver tissue regeneration and hepatocyte restoration in the groups treated with the extracts. These findings corresponds to a similar study (Ukpabi *et al.*, 2018).It has been proven that saponins show modulatory effects on transaminases in hepatocytes of rats against liver injury, which could be as a result of their antioxidant mechanism of action [18]. Therefore, ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* which contains saponins, may have stabilized the membrane integrity and prevented these enzymes' leakage into blood circulation [12]. This indicated the protective activity of ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* revealing that the ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* has the ability of protecting not only the structural integrity of the hepatocellular membrane but also the phenylhyrazine -damaged cells. Serum ALP and GGT activities increased significantly ($P<0.05$) in the anaemic control group relative to the normal control, but treatment with the leaf-extracts and the standard multivitamin significantly ($P<0.05$) decreased the activities of these liver function parameters (Figure 4 - 5). It has been reported that alterations in enzymes activities in the serum directly indicates major pathologic changes in cell

membrane permeability or hepatic cell rupture, a signal of underlying pathological process [7]. ALP in the cellular external membrane plays the major role in phosphate metabolism and it prevents the external membrane from being damaged. Effective control of alkaline phosphatase activity points towards an early improvement in secretory mechanism of hepatic cells [11]. Increase in plasma level of ALP is due to increased synthesis of the enzyme in the presence of increasing biliary pressure. A rise in ALP levels can indicate liver trouble if GGT levels are also elevated. Patients with predominantly elevated ALP and GGT are described as having cholestatic disease [13]. Elevation of ALP occurs as a result of obstructed bile flow of either the intra-hepatic or extra-hepatic biliary tree. Patients with elevated ALP levels commonly have granulomatous liver disease. Causes of elevated ALP and GGT levels include primary biliary cirrhosis, fatty liver, alcoholic liver disease, liver inflammation from medications and certain herbs, liver tumors and gallstones or gall bladder problems [8]. Increased GGT activity might reflect an increased consumption of glutathione, an intracellular antioxidant, and a marker of oxidative stress. Therefore, increased GGT activity may reflect oxidative stress in patients with nonalcoholic fatty liver disease (NAFLD) [16,19,21]. Patients with elevated GGT levels may have more inflammatory reactions in the liver, including oxidative stress-related fat accumulation and hepatocellular damage. Increased fatty deposition in the liver induces hepatocellular inflammation and damage, which results in GGT synthesis and ALT elevation [11,15,18]. The concentration of TP indicates the amount of albumin and globulin in blood. As proteins are necessary for growth, development, and health, the level of TP is highly related to symptoms like weight loss [26]. However, albumin performs an important function in binding many substances, reducing their availability and toxic actions (Ibiam *et al*., 2013). The result of the present study showed significant ($P<0.05$) decrease in the levels of serum total protein in the anaemic rats relative to the normal control (Figure 6). This was significantly ($P<0.05$) increased on treatment with the leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* and the standard multivitamin (Figure 6). The reduction of total protein may be due to proteolysis, increased metabolism under toxicant stress as well as reduction in protein synthesis due to phenylhydrazine-induced damages to hepatocytes responsible for liver protein synthesis [11]. The normal total protein and albumin levels are associated with appropriate liver function [7], The reduction in the serum total protein, regarding the phenylhydrazine intoxicated group, might be due to liver damage. However, this was reversed on treatment with the leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* and the standard multivitamin (Figure 6). Tests for total bilirubin (TB) and conjugated/direct bilirubin are used to diagnose liver dysfunction and/or monitor the progression of treatment of liver disease [6]. There was significant ($P<0.05$) increase in the levels of serum total bilirubin in the anaemic rats relative to the normal control. Treatment with the crude ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* at 100, 200 and 400 mg/kg body weight and the standard multivitamin significantly ($P<0.05$) reduced the serum levels of this parameter in a dose dependent manner (Figure 7).

Total bilirubin is considered as one of the accurate tests of liver functions since it reflects the ability of this organ to take up and process bilirubin into bile (Arias 2012). Thus the high levels of total bilirubin in the phenylhydrazine induced treated rats could have been caused by phenylhydrazine toxicity (Figure 7). In the present study, increased bilirubin levels may have been caused by breakdown of erythrocytes and other haem containing proteins, myoglobin and cytochromes. The haem (from porphirin) of the haemoglobin molecule is separated from the globin and haem is converted mainly in the spleen to bilverdin which is

reduced to bilirubin [8]. This is also in tandem with the observed increase in total bilirubin (TB) and conjugated / direct bilirubin levels due to exposure to phenylhydrazine [11]. It also corroborates the decrease in RBC, haemoglobin and packed cell volume levels due to exposure to phenylhydrazine as observed in this current study (Table 1). There was significant ($P<0.05$) increase in the levels of serum direct/conjugated bilirubin in the anaemic rats relative to the normal control. Treatment with the crude ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei* at 100, 200 and 400mg/kg body weight and the standard multivitamin significantly ($P<0.05$) reduced the serum levels of these parameters in a dose dependent manner (Figure 8).

The binding (conjugation) of bilirubin protects the newborn child against the toxic action of the substance, preventing it from penetrating the blood-brain–barrier. The binding ability of albumin to drugs is reduced in hypoalbuminaemia when binding sites are blocked by various metabolite [9]. Although these hepatoprotection cannot be ascribed to a particular chemical constituent of the extracts, some of their phytochemicals such as flavonoids and phenols are known to poses antioxidant properties. Effective control of Bilirubin levels points towards an early improvement in secretory mechanism of hepatic cells [18]. The observed results were corroborated by histopathological examination of the liver. In the present study, Photomicrograph of group 1 control section of liver showed normal hepatic architecture with central vain (CV) hepatocyte(H) and well perfussed cytoplasm (C) (Figure 14). According to the histopathological assessment, liver injury in exposed rats was in sharp contrast with the controls (Figure 9). The liver photomicrograph of group 2 section of liver induced with anaemia showed severe degeneration with severe focal aggregate of intra hepatic inflammation (FAIHI) (Figure 9), indicating the toxic effect of phenylhydrazine. These histopathological results regarding liver pathology were quiet contrasting compared to the control group. A variety of toxic effects of PHZ have been described, including haemolytic anaemia, hypoxia, inflammation, alterations in the liver, kidney, central nervous system, autoimmune disturbances and cancer PHZ is known to shorten life-span of red blood cells [8]. It has been shown that in chronically diseased liver, some cells are activated by factors released by the liver hepatocytes and Kupffer cells, proliferate, and acquire the features of myofibroblasts, with or without the lipid droplets [9]. Histopathological findings also revealed the protective effects of ethanol leaf-extracts of *Telfairia occidentalis* and *Mucuna poggei*. Photomicrograph of group 3 section of liver induced with anaemia and treated with 0.1 ml/ kg of standard multivitamin drug) showed moderate healing with mild focal aggregate of intra hepatic inflammation (FAIHI) (Figure 10). Photomicrograph of group 4 section of liver induced with Anaemia and treated with 400mg/kg of MP1 showed moderate healing with mild focal aggregate of intra hepatic inflammation (FAIHI) (Figure 11). Photomicrograph of group 5 section of liver induced with Anaemia and treated with 200mg/kg of MP2 showed mild healing with moderate portal aggregate of inflammatory cell (PAIC) (Figure 12).Photomicrograph of group 6 section of liver induced with Anaemia and treated with 100mg/kg of MP3 showed mild healing with moderate intra hepatic inflammation(IHI) and cytoplasmic ground glass appearance(CGGA) (Figure 13). Photomicrograph of group 7 section of liver induced with Anaemia and treated with 400mg/kg of TO1 showed moderate healing with mild aggregate of intra hepatic inflammation(IHI) (Figure 14). Photomicrograph of group 8 section of liver induced with Anaemia and treated with 200mg/kg of TO1 showed mild healing with moderate congestion of the central vain (CCV)and moderate aggregate of inflammatory cell (AIC) (Figure 15) arround the congested vain. Photomicrograph of group 9 section of liver induced with Anaemia and treated with 100mg/kg of TO1 showed moderate healing with mild portal aggregate of inflammatory cell (PAIC) (Figure 16). Generally, the liver showed congestion, edema, atrophy, and degeneration of hepatocytes, 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.

CONCLUSION

Ethanol leaf-extracts of *Mucuna poggei* and *Telfairia occidentalis* possess haemoprotective and hepatic tissue regeneration ability in phenylhydrazine induced anaemic Wister rats. These findings support the claim by traditional herbalists that these leaf-extracts are effective in the treatment of anaemia and in ameliorating hepatic complications. Also, the current study will take a lead in the discovery of the mechanism of action of phyto-active agents with little or no side effects from both plants.

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