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Toxicological Evaluation and Possible Reversal of Diabetic Toxicological Complications by PHF5 an Antidiabetic Herbal Formula in Wistar Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author ESI carried out animal study, managed the analyses and wrote the first draft of the manuscript. Authors NOP and EMP wrote the protocol and also managed the analyses of the study. Author CIG carried out animal study, managed literature search and wrote the manuscript. Author COH and WEC carried out literature searches and did statistical analyses. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Medicinal potentials of herbs can be amplified greatly through synergism in a poly-herbal formulations. Toxic propensities of a plant can be masked out in poly-herbal preparations thus enhancing effectiveness. The aim of this study was to evaluate the potential toxicological effect and toxicity ameliorating properties of a poly-herbal formula (PHF5) prepared in a particular ratio from the leaves of *Ocimmum gratissimum, Vernonia amygdalina, Gongronema latifolium, Gnetum africanum* and *Aloe barbadensis* against alloxan-induced diabetic male rats.
Methods: Thirty-six (36) Wistar rats divided into 6 groups with 6 animals per group were used for this study. Group 1 served as normal control animals; group 2 had the diabetic rats treated with PHF5 (75 mg/kg bw); group 3, diabetic rats treated with PHF5 (150 mg/kg bw); group 4, diabetic rats treated with PHF5 (300 mg/kg bw); group 5, diabetic rats not given any intervention, group 6

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diabetic animals treated with Glibenclamide (5 mg/kgbw). The induction of diabetes was done intraperitoneally using alloxan monohydrate (100 mg/kg bw). Administration of PHF5 was done orally for five weeks.
Results: Acute toxicity studies of PHF5 did not show any toxic symptoms in animals that received the PHF5 at up to 5000 mg/kg bw dose. The elevated liver (TP, AST, ALT and ALP) and kidney (BUN and Creatnine) markers in the diabetic animals were lowered significantly in the PHF5-treated animals.
Conclusion: The findings of this study suggests that PHF5 possess protective properties against hepatotoxicity and diabetic nephropathy and that the extract is quite safe for consumption

Keywords: Toxicity; Wistar rats; poly-herbal formula; antidiabetic; alloxan monohydrate.

1. INTRODUCTION

Diabetes mellitus is a serious debilitating disease of carbohydrate metabolism. It is characterised by chronic pathologic elevation in blood glucose; a condition known as hyperglycemia. Diabetes results as a result of complete or partial deficiency of insulin or receptor defect/resistance that makes the system unable to utilize available insulin[1] Acute hyperglycemia seen in diabetes a precursor to a range of an increasing array of pathologic disorders leading to damage of vital organs such as heart, eyes, blood vessels, kidneys, and nerves [2,3]. The most predisposing factor for diabetes is of genetic origin, however environmental factors such as increased consumption of carbohydrate rich meals and sedentary lifestyle are important triggers of diabetes.

In its early stages, diabetes is characterized by chronic hyperglycemia and hyperinsulinemia. Its progression involves a complex network of interacting cellular and physiological alterations leading to β cell failure. Glucotoxicity and lipotoxicity are the most common consequencies of this failure [4].

Glucotoxicity arises from excessive hyperglycaemia. The excess sugar drives glycation reactions and the mitochondrial electron transport chain, producing macromolecule-damaging reactive oxygen species (ROS), at levels beyond the anti-oxidation capacity of the cell. The ensuing oxidative stress impairs insulin synthesis and secretion, and initiates a cascade of cellular events that ultimately lead to apoptosis [5].

Lipotoxicity, on the other hand, results in part from the unresponsiveness of adipocytes to insulin, negating the ability of this hormone to stimulate uptake of non-esterified fatty acids (NEFA) and to inhibit lipolysis of endogenous triacylglycerol (TG) to NEFA. Excess plasma NEFA impairs insulin secretion by β cells, stimulates gluconeogenesis by liver, and inhibits glucose disposal by skeletal muscle, further exacerbating hyperglycemia [6]. Impaired TG storage into adipocytes facilitates the formation in the bloodstream of small, cholesterol ester-poor, TG-rich low-density lipoprotein (LDL) particles. Hyperglycemia promotes glycation of these particles, a modification that extends their half-life in circulation. These particles are prone to oxidation and are potent initiators of atherogenesis and its vascular damages. Diabetes-associated neuropathy, retinopathy, and nephropathy are some of the consequences of these damages [7]. Summarily insulin resistance and impaired insulin secretion lead to hyperglycemia, hyperlipidemia and to an increase in hepatic glucose. The chronic hyperglycemia and abnormalities in serum lipids of diabetics is associated with long-term downstream damages seen in chronic diabetes [8].

The epidemic spread of diabetes and identification of new therapeutic avenues in the treatment of all pathological aspects of this disorder remain a major challenge for current biomedical research. Wide varieties of pharmacological drugs are being used for diabetic treatment currently but are not always satisfactory in maintaining normal level of blood glucose and avoiding late stage diabetic consequences. Equally worrisome is the attendant toxic side effects of these drugs, thus the increasing demand for natural products with anti-diabetic properties and less side effects [9].

This study proposes to investigate the anti-diabetic potentials of a poly-herbal formula of five plants known to possess anti-hyperglycaemic effects individually. These include: *Vernonia amygdalina, Gongronema latifolium, Allium sativium, Ocimum gratissimum* and *Aloe barbadensis*. Preliminary research on this herbal cocktail showed a great promise of synergistic

antidiabetic effect [10] hence the need to further study the mechanism(s) of action(s). It is equally important to study the shelf life potency of the powdered extract over a period of time.

Beneficial effects of this herbal cocktail individually and collectively might be by correcting altered glucose metabolism, maintaining integrity and function of β-cells, insulin secreting activity and enhancing glucose up take and utilization. The aim of this study was to evaluate the potential toxicological effect and toxicity ameliorating properties of a poly-herbal formula (PHF5) prepared in a particular ratio from the leaves of *Ocimmum gratissimum, Vernonia amygdalina, Gongronema latifolium, Gnetum africanum* and *Aloe barbadensis* against alloxan-induced diabetic male rats.

2. MATERIALS AND METHODS

2.1 Plant Extract Preparation

Leaves of AB, GA, GL, VA and OG were purchased from a rural market in Umuahia, Abia State. The plants leaves were picked, sorted, washed with distilled water and air dried under shed to a constant weight. They were pulverized into a powder using an ETKAL 868 electric power blender and then stored in air-tight plastic containers with appropriate labelling.

2.2 Preparation of PHF5

The powders of the different plants VA, GL, OG, AS and AB were mixed together in a determined ratio to derive the poly-herbal formula (PHF5) used for this study. The PHF5 was soaked in hot water and filtered after two minutes. The filtrate was freshly prepared each time and administered orally on the animals.

2.3 Animals

Male Wistar rats, weighing 80 to 120g were purchased from an animal farm at the University of Nigeria Nsukka and used for this study. The animals were housed in aluminum cages (6 animals per cage) in clean conditions at ambient room temperature of 25^{o}C (± 2^{o}C) with 12-hour light and 12-hour dark cycle. The Wistar rats were acclimatized for seven days before commencement of the experiment. They were fed standard feed and water *ad libitum*. The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout the duration of this study. The induction of diabetes was done intraperitoneally using alloxan monohydrate (100 mg/kg bw).

2.4 Experimental Design

The induction of diabetes mellitus was achieved by dissolving alloxan monohydrate in normal saline and this was administered to the animals at a dose level of 100 mg/kg intraperitoneally. The fasting blood glucose level of the animals was investigated 72 hours after administration of the drugs to confirm induction. A minimum fasting blood glucose concentration of 140 mg/dL in the animals was the yardstick for selection of diabetic animals (Weir et al. 1981). Thirty- six (36) Wistar rats divided into 6 groups with 6 animals per group were used for this study. Group 1 served as normal control animals; group 2 had the diabetic rats treated with PHF5 (75 mg/kg bw); group 3, diabetic rats treated with PHF5 (150 mg/kg bw); group 4, diabetic rats treated with PHF5 (300 mg/kg bw); group 5, diabetic rats not given any intervention, group 6 diabetic animals treated with Glibenclamide (5 mg/kg bw). The administration of PHF5 was done orally via the intubation tube.

2.5 Acute Toxicity

Acute toxicity of PHF5 was carried out in accordance with the guidelines set by the Organization for Economic Cooperation and Development (OECD). Three animals per dose were used for this experiment. The rats were fasted overnight and orally fed with PHF5 at dose levels of 200, 400, 800, and 1000 mg/kg body weight. The animals were observed for their behavioral (alertness, restlessness and irritability), response to touch, response to pain and spontaneous activity, rate of urination and defecation for 24 hours. The animals were monitored for fourteen more days for mortality.

2.6 Biochemical Metabolic Parameters

Administration of extracts lasted for 35 days. At the end of the administration period, the animals were anaesthetized and blood samples collected via cardiac puncture. The collected samples were stored in clean sample tubes and centrifuged at 4000g for 15 minutes. Plasma aliquots were used to estimate levels of biochemical parameters following biochemical principles as contained in the Randox kits.

2.7 Statistical Analysis

Statistical significance between the groups were determined using One-way ANOVA followed by Duncan's post-hoc test. Normalty test was

carried out by Shapiro-Wilk test and accepted at p value > 0.05. Levene's test was used to determine homogeneity of variance ($p<0.05$). Data was represented as means ± standard deviation (M ± SD). Significant values were accepted at values < 0.05. Statistical analyses of data were performed using SPSS version 22.

3. RESULTS

Data for liver function markers is shown in Table 2. The diabetic control animals showed a significantly reduced total protein levels compared to the normal animals. At 300mg/kg, the PHF5 significantly ($P<0.05$) increased total protein in the animals compared to diabetic control animals. ALT and AST activities were significantly elevated in the diabetic control animals compared to the normal animals. At all the administered doses, (75mg/ kg, 150 mg/kg and 300mg/kg) the PHF5 significantly decreased AST and also lowered ALT activity at doses of 150 mg/kg and 300mg/kg compared to the diabetic control animals. The diabetic control animals showed a significant reduction in ALP activity when compared to the normal control animals, whereas the poly-herbal formula at 75mg/kg and 150 mg/kg significantly elevated ALP activity when compared to the diabetic control animals. The Glibenclamide-treated animals also showed significantly higher ALP activity compared to the diabetic control. ALB concentration was elevated significantly in diabetic rats compared to the normal animals. This was however reversed significantly in the animals treated with PHF5 at low doses.

The effect of PHF5 and Glibenclamide on BUN and creatinine of diabetic and PHF5–treated animals are presented in Table 3 above. The diabetic animals showed a significant ($P < 0.05$) increase in creatinine concentration compared to the normal animals. PHF5 at 75mg/kg, 150 mg/kg and 300mg/kg as well as Glibenclamide significantly ($P < 0.05$) lowered creatinine levels compared to the diabetic control animals. The diabetic control animals showed a significant elevation of BUN when compared to the normal animals while the PHF5 and Glibenclamide were able to lower BUN close to that of the normal animals.

Table 1. Effect of PHF5 on liver function markers in Alloxan-induced diabetic rats

Treatment	Liver function markers					
	Total protein (g/dL)	**AST (U/L)**	**ALT (U/L)**	**ALP (U/L)**	**ALB (g/dL)**	**BIL (mg/dL)**
Normal control	6.22±1.04^{ab}	119.20±5.52^{a}	22.85±3.89^{a}	97.37±6.97^{a}	3.04±0.59^{ab}	0.64±0.24^{a}
PHF5 75 mg/kg	6.00±0.74^{a}	140.66±8.73^{c}	39.09±2.41^{b}	191.81±14.64^{c}	3.22±0.31^{ab}	0.85±0.45^{a}
PHF5 150 mg/kg	6.38±0.55^{bc}	112.82±1.44^{a}	23.34±5.89^{a}	184.35±5.74^{c}	2.78±0.49^{ab}	0.79±0.24^{a}
PHF5 300mg/kg	6.73±0.06^{c}	103.30±2.14^{a}	30.17±9.42^{a}	172.19±1.43^{bc}	3.70±0.26^{bc}	0.96±0.62^{a}
Diabetic control (Alloxan)	5.70±1.04^{ab}	164.84±0.97^{c}	33.43±1.72^{b}	192.81±8.27^{b}	3.96±0.25^{c}	0.96±0.13^{a}
Glibenclamide	5.71±0.79^{ab}	109.27±7.19^{a}	17.21±2.08^{a}	159.68±7.24^{b}	2.94±0.35^{a}	0.79±0.06^{a}

PHF5: Poly-herbalformular. Values are expressed as mean±SEM (n=6). Values between groups with different superscripts are significantly (P<0.05) different (one-way ANOVA followed by Duncan's Post Hoc test), AST: Aspartate Transaminase, ALT: Alanine Transaminase ALP: Alkaline Phosphatase, ALB: Albumin, BIL:Bilirubin

Table 2.Effect of PHF5on Urea and Creatinine in Alloxan-induced diabetic rats

Treatment	BUN (mg/dL)	Creatinine (mg/dL)
Normal control	24.05±5.36^{a}	0.54±0.11^{ab}
PHF5 75mg/kg	27.31±7.09^{a}	0.77±0.06^{c}
PHF5 150 mg/kg	24.24±6.70^{a}	0.79±0.07^{c}
PHF5 300mg/kg	24.80±13.80^{a}	0.720±0.12^{bc}
Diabetic control (Alloxan)	32.49±1.39^{b}	0.94±0.50^{d}
Glibenclamide	32.78±3.80^{a}	0.89±0.28^{a}

PHF5: Polyherbalformular. Values are expressed as mean±SEM (n=6). Values between groups with different superscripts are significantly (P<0.05) different (one-way ANOVA followed by Duncan's Post Hoc test). BUN: Blood Urea Nitrogen

GROUP 1 : CONTROL

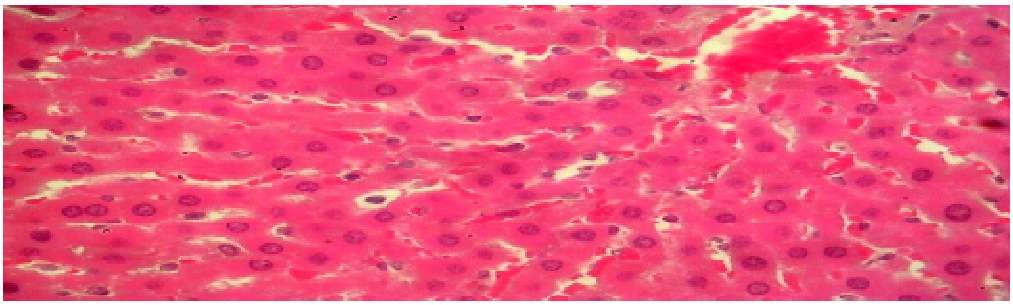

Fig. 1 . LIVER: Sections of the liver presented in this group showed the normal histomorphology of the rodent liver. The sections showed normal hepatocytes arranged in interconnecting cords in a radial manner, around the central veins. The hepatic cords are separated by the hepatic sinusoids and radiates towards the periphery of the hepatic lobules where they connect with the components of the portal triad (hepatic artery, hepatic vein and bile duct). Central vein (V), H&Ex400

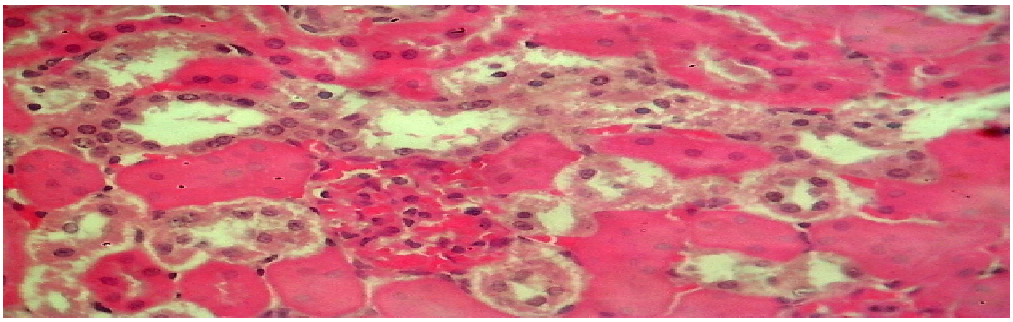

Fig. 2 :KIDNEY Sections of the kidney presented in this group showed a moderate widespread degeneration and necrosis of the epithelial lining cells of the renal tubules (arrow) of the cortex and outer medullar. The affected renal tubules show epithelial lining cells with marshy cytoplasm and indistinct cell boundarieswith some cells exhibiting nuclear pyknosis (black arrow), Glomerulus (G). H&Ex400

GROUP 2: PHF 75mg

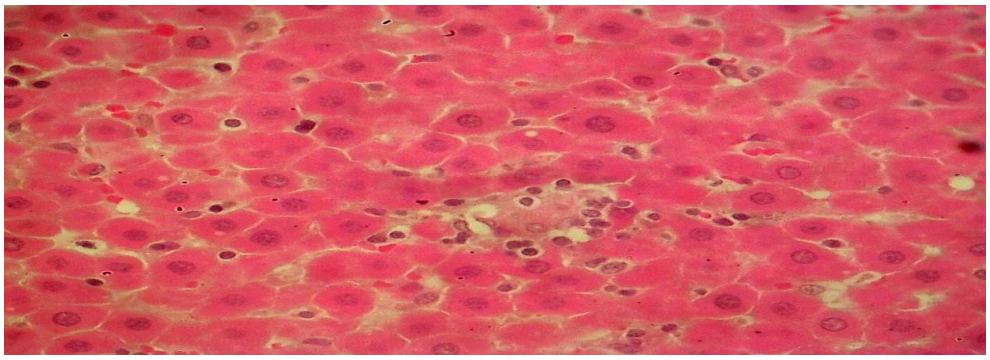

Fig. 3. LIVER: The sections of the liver presented in this group showed a random widespread/multifocal necrosis of the hepatocytes (arrow) with infiltration of mononuclear leukocytes. Hepatic cord dissociation with individualization of the hepatocytes was also observed. The hepatocytes appear singly or in clusters instead of being arranged in interconnecting cords, H&Ex400

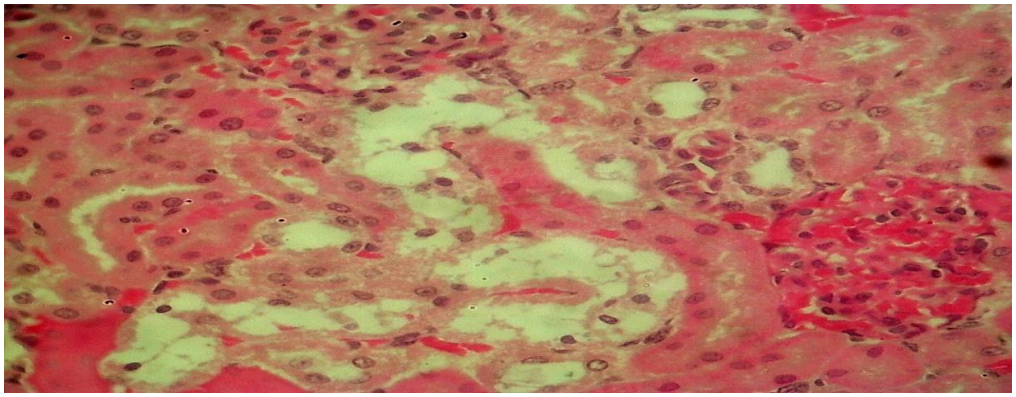

Fig. 4. KIDNEY: Section of the kidney presented in this group showed a marked, widespread degeneration and necrosis of the epithelial lining cells of the renal tubules (arrow), Glomeruli (G). H&E x400

GROUP 3: PHF 150mg

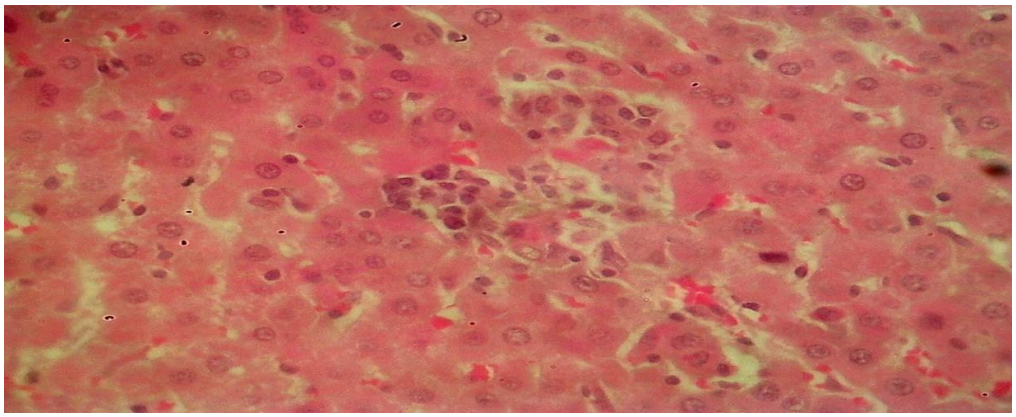

Fig. 5. LIVER: Sections of the liver presented in this group showed moderate multifocal hepatocellular necrosis with mild infiltration of mononuclear leucocytes (arrow), H&Ex400

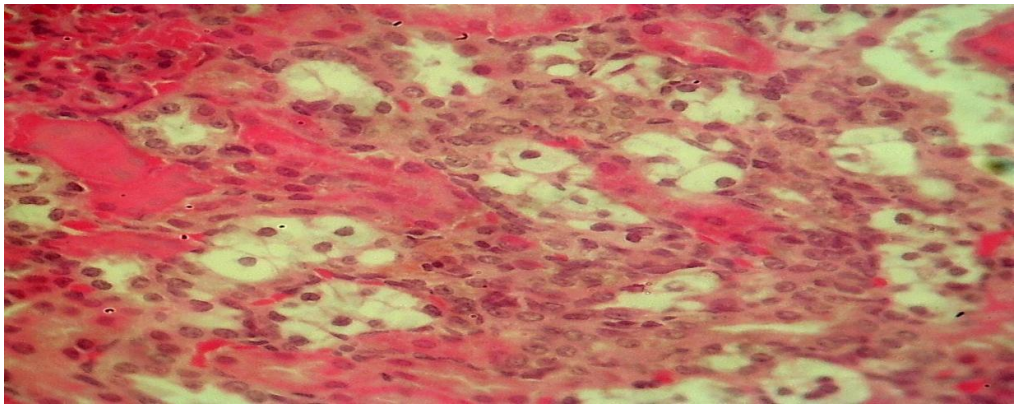

Fig. 6. KIDNEY: Section of the kidney presented in this group showed a marked, widespread degeneration and necrosis of the epithelial lining cells of the renal tubules (white arrow) admixed with segments of renal tubular regeneration evidenced by clusters of proliferating epithelial cells (Blue arrow). Glomeruli (G). H&E x400

GROUP 4: PHF 300mg

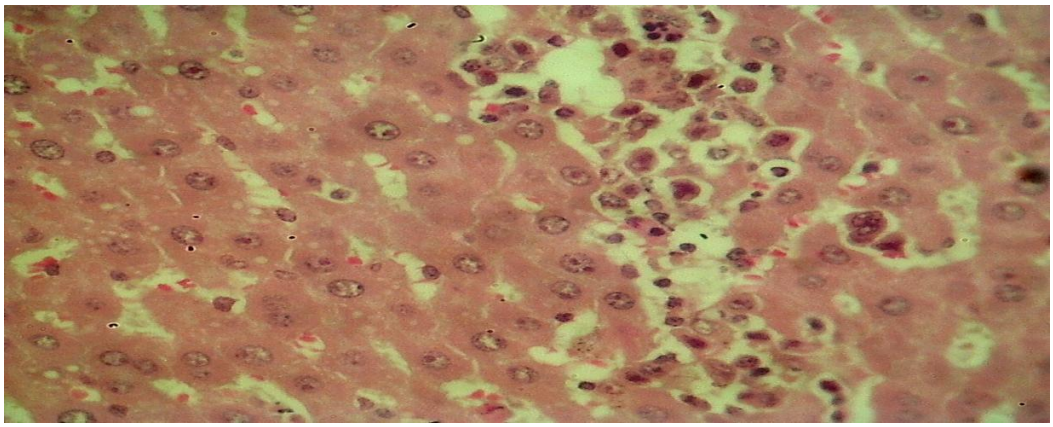

Fig. 7. Sections of the liver presented in this group showed moderate multifocal hepatocellular necrosis with mild infiltration of mononuclear leucocytes (arrow), H&Ex400

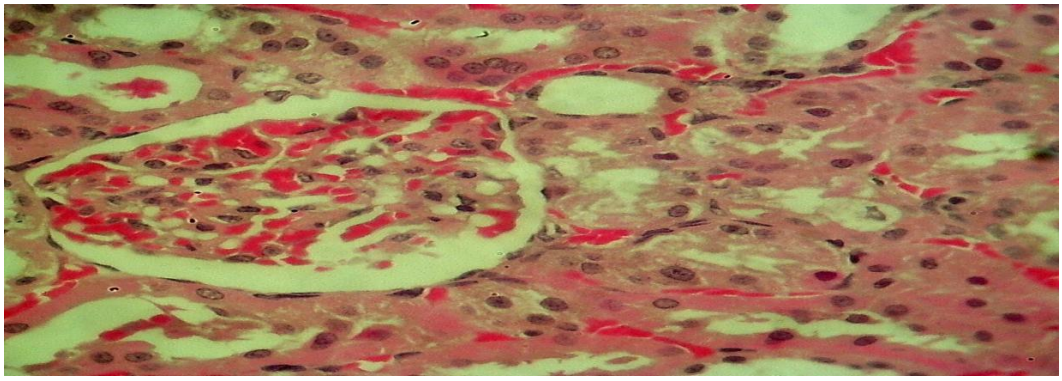

Fig. 8. KIDNEY: Sections of the kidney presented in this group showed a mild multifocal degeneration of the renal tubular epithelial cells. The affected renal tubular epithelial cells appear swollen, with foamycytoplasm (arrow). Glomerulus(G), H&Ex400

GROUP 5: UNTREATED DIABETIC ANIMALS

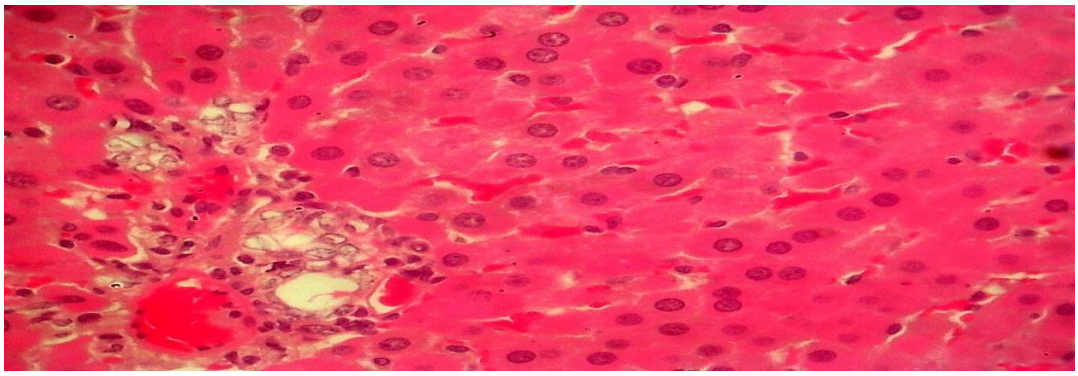

Fig. 9. Sections of the liver presented in this group showed severe multifocal hepatocellular necrosis with mild infiltration of mononuclear leucocytes. See group 1 for details. Hepatic vein (HV); Bile duct (BD), H&E x400

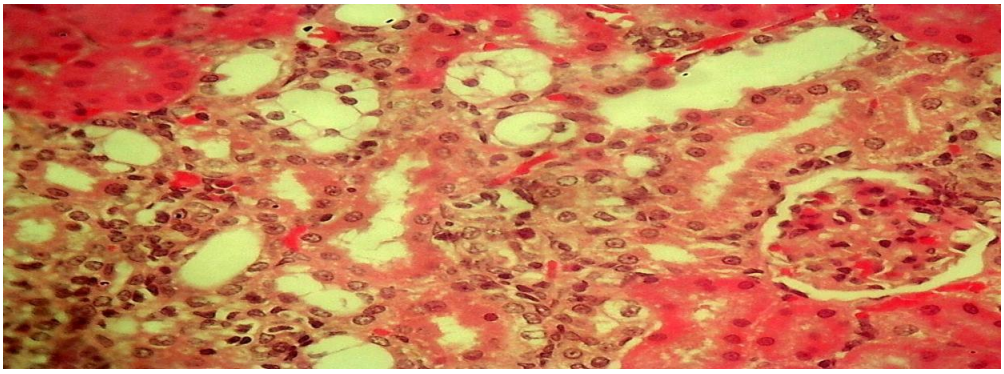

Fig. 10. KIDNEY: Section of the kidney presented in this group showed a marked, widespread degeneration and necrosis of the epithelial lining cells of the renal tubules (white arrow) admixed with segments of renal tubular regeneration evidenced by clusters of proliferating epithelial cells (Blue arrow). Glomeruli (G), H&E x400

GROUP 6: DIABETIC ANIMALS + STANDARD DRUG

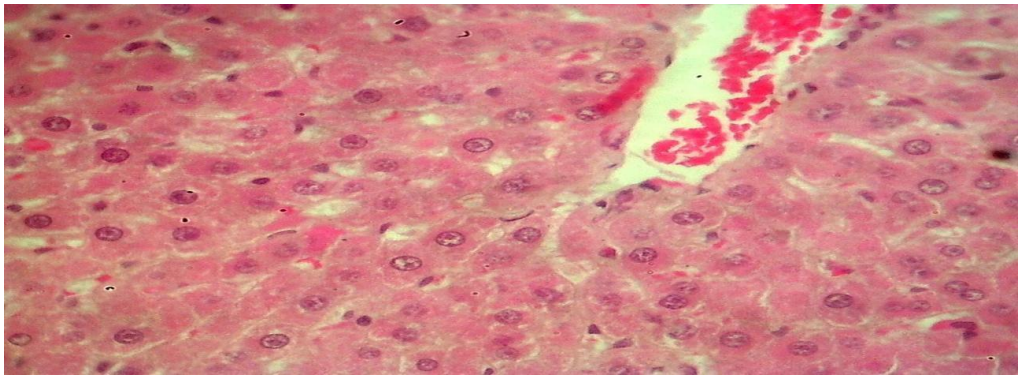

Fig. 11. LIVER: Sections of the liver presented in this group showed the normal histomorphology of the liver. See group 1 for details. Central vein (V), H&E x400

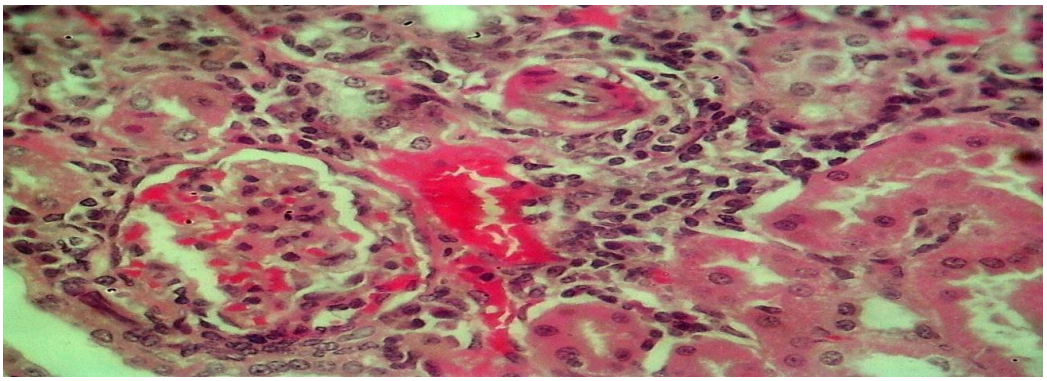

Fig. 12. KIDNEY: Section of the kidney presented in this group showed a marked, widespread degeneration and necrosis of the epithelial lining cells of the renal tubules admixed with segments of renal tubular regeneration evidenced by clusters of proliferating epithelial cells. In addition, multifocal infiltration of the renal interstitium by mononuclear leucocytes (blue arrow) and thickening of the Bowman's capsules (white arrow) were observed. Glomeruli (G), H&E x400

4. DISCUSSION

Poly herbal formulations are becoming very important in the management of diseases as a result of positive synergistic effects. Being natural compounds, they are not usually toxic and can be procured very cheaply. Herbs play important roles in the management of diabetes and many other common health disorders [11,12].

This study investigated possible toxicological potentials of different therapeutic doses a poly-herbal formulation (PHF5) in normal and diabetic rats. The hyperglycemia induced by alloxan caused significant reduction of total protein as well as significant elevation of plasma levels of AST and ALT. The reduction in total protein level might suggest a reduction in the rate of synthesis of protein or an increased rate of protein breakdown. These play a major role as important clinical markers of diabetes [13]. The decrease in total protein is also suggestive of microproteinuria which has been reported to precede the development of overt nephropathy in diabetes mellitus [14].

Plasma concentrations of aspartate transaminase (AST), alkaline phosphatase (ALP) and alanine transaminase (ALT) are important indicators for assessing liver injury. The liver is one of the primary organs prone to the adverse impact of oxidative stress as a result of hyperglycaemia [15]. Remarkable increases in plasma levels of ALT and AST in diabetic control rats suggest damage to hepatic tissues causing the release of these enzymes from hepatic cells into the plasma. Administration of PHF5 to diabetic rats was able to attenuate the damage caused to the liver as evidenced by the significant reduction in enzyme activity in the PHF5- treated animals.

Plasma concentration of BUN and creatinine are considered indicators of renal function. BUN, is a non-protein nitrogenous (NPN) by-product of protein metabolism. Amino acids that are products of protein breakdown undergo a deamination reaction to produce ammonia. The ammonia is subsequently converted to urea by liver enzymes [16]. Urea concentration therefore depends on the intake of protein, the capacity of the body to breakdown protein, and the ability of the renal system to adequately excrete urea. This study showed elevation in BUN and creatinine as seen in diabetic control animals in this study. A reduced glomerular filtration rate of the kidney will result to an increased plasma concentration of creatinine, while an elevation in BUN can be a consequence of a decreased renal excretion both of which are regarded as biomarkers of renal dysfunction. Reports have shown that hyperglycaemia is a major cause of progressive renal dysfunction [17,18]. The PHF5 was able to reverse the impact of the alloxan-induced damage to renal function by lowering the concentrations of BUN and creatinine to near normal levels. This could be attributed to the nephro-protective properties that have been reported of the different plant herbs used in preparation of the PHF5 [19-22]. Histological examination of tissues concurred with the result of the biochemical analysis and further strengthen the suggested properties of the extract to ameliorate diabetic toxicity in different organs of the body.

5. CONCLUSION

Data from this study demonstrate the toxicity attenuation effect of the poly-herbal formulation PHF5 evidenced by significant decreased blood levels of ALT, AST, ALP, urea and creatinine in the rat groups administered extracts compared to the untreated diabetic control. Results of this study suggest that the poly-herbal formula is very safe for consumption and beneficial in combating diabetic toxicity in body organs.

ETHICAL APPROVAL

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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