Original Research Article

EFFECT OF POLY-HERBAL FORMULA (PHF5) ON HEPATOPROTECTIVE AND BIOCHEMICAL PARAMETERS OF ALLOXAN-INDUCED DIABETIC WISTAR RATS.

ABSTRACT

Background: The current estimates by World Health Organization revealed that near 2030, the number of diabetic patients will reach up to 370 million in the world wide . It would be one of the most common degenerative illnesses in human beings in future that needs to seek urgent solution. Plants have been the major source of medicine since the ancient time. **Purpose:** This study evaluated the hepaprotective action of Ocimum gratissimum, Gnetum africanum, Gongronema latifolium, Vernonia amygdalina and Aloe Barbadensis leaf extracts and their effects on biochemical parameters of induced alloxan diabetic wister rats. Standard methods were used in the extraction process. **Methods;**Thirty (30) male Wistar rats weighing (80_120) grams were obtained and they were kept in animal house to acclimatize for two weeks prior to the experiment. The rats were divided into six groups containing five rats each. Group 1 served as normal control, group 2 had the diabetic rats treated with PHF5 (75 mg/kg bodyweight); group 3 contained diabetic rats treated with PHF5 (150 mg/kg bodyweight); group 4 contained diabetic rats treated with PHF5 (300 mg/kg bodyweight); group 5 had diabetic rats not given any intervention, group 6 contained diabetic rats treated with Glibenclamide (5 mg/kg bodyweight). The rats were induced via intra peritoneal by using alloxan monohydrate (100 mg/kg bodyweight). Extracts of PHF5 were administered to the rats orally for eight weeks, after which rats were sacrificed by cervical dislocation under light ether anaesthesia. Blood was collected for biochemical evaluation using standard techniques (Randox kits). Fasting blood glucose level was checked weekly. **Results;** Acute toxicity studies of PHF5 revealed no toxicity to the animals that received the PHF5 dose up to 1000 mg/kg bodyweight. The decreased liver (Aspartate amino transferase, Alanine Transferase and Alkaline Phosphatase) marker enzymes in the diabetic rats were significantly ($p<0.05$) lowered in the PHF5 treated rats and found to be within the normal range while total protein was significantly higher showed no effect on the liver total protein. **Conclusion;** It is concluded that this study suggest that treating alloxan induced diabetic rats with poly herbal formulated (PHF5); Ocimum gratissimum, Gnetum africanum, Gongronema latifolium, Vernonia amygdalina, and Aloe Barbadensis leaf extracts in different doses of mg/kg enhanced hepaprotective protection against body damage.

Keywords: *Alloxan monohydrate, Diabetes, hepaprotective, Poly-herbal formula, biochemical parameters, Alanine transferase, Aspartate amino transferase, Alkaline phosphatase and Glibenclamide.*

1. INTRODUCTION

Diabetes mellitus (DM) is one of the most common forms of metabolic and life threatening disease that affect the world wide [1]. It is a chronic disease caused by changes in the metabolism of carbohydrates, proteins, and lipids due to absolute or insufficient insulin deficiency [2,3]. Diabetes mellitus (DM) leads to a risk of several other diseases, many of which are debilitating and followed by an increasing risk of death

[3]. Abnormalities observed in DM patients could progress to lesions that include nephropathy, neuropathy, retinopathy and angiopathy [3]. Early-stage symptoms of diabetes are hyperglycemia with hyperinsulinemia as a result of the insensitivity of tissues to insulin. DM are characterized by physiological and cellular changes that result in the demise of beta (β) cells due to the progression of the disease [4]. Failure of β -cells result from glucose toxicity and lipid toxicity, and an excessive glucose uptake causes glucose toxicity by the islet beta (β)-cells [4]. Elevated sugar levels trigger glycation reactions and reactions in the electron transport chain, resulting in an imbalance in the cell's antioxidant capacity due to an increase in the production of reactive oxygen species [5]. The consequent oxidative stress leads to a decrease in insulin production and secretion, initiating a sequence of cellular events that ultimately lead to death [5]. In addition to the burden of living with diabetes, it is associated with high morbidity and mortality rates and therefore accounts for a large part of the public health care expenses. Intensified diabetes research is therefore required to improve our knowledge and ability to prevent and treat diseases.

Evaluation of plant products to treat diabetes is of high interest as they contain many bioactive substances with therapeutic potentials [6]. Several plants are efficient ameliorators of stress associated with diabetes, and many medicinal plants have already been tested for their hepatoprotective properties, these effects remain to be investigated in various other medicinal plants [6].

Ocimum gratissimum, also known as basil from the Family of Lamiaceae, is a culinary herb with a pungent sweet smell [7]. The leaves are often used fresh in cooking or added at the last minutes, as cooking for a long period might destroys the flavour quickly [8]. Studies have reviewed that basil oil possess potent antioxidants, anticancer, antiviral and antimicrobial properties [9]. The leaves extract also been reported to possess antidiabetic properties in alloxan-induced diabetic rats [10,11].

In the South-Western and South-Eastern parts of Nigeria*, Gnetum africanum* (eru or African jointfir), *Gongronema latifolium*, commonly called "utazi" and "arokeke," and *Vernonia amygdalina*, commonly called bitter leaf" in English because of its bitter taste, are mainly used as a vegetable for soups and stews [12,13]. *Gnetum africanum* is a good source of both essential and non-essential amino acids, which contains high levels of leucine, aspartic acid, and glutamic acid, with low levels of histidine and cysteine. The leaves can treat nausea, sore throats, or dressing for warts [14].

Cold concoctions of *Vernonia amygdalina* are used to treat malaria, intestinal parasites, diarrhoea, and stomach pain [14]. A concoction of this plant is also used for malarial fever, schistosomiasis, amoebic dysentery, and many other intestinal parasites and stomach pains in many African ethnic groups. [15].

Traditionally folk medicine uses *Gongronema latifolium* [16]. Digestive problems such as dyspepsia, anorexia, colic and stomach pain, constipation, dysentery, intestinal worms, hyperglycemia, and hypertension are commonly treated with an infusion or decoction of the whole plants (the leaves, stems, and roots) [17, 18, 19,20, 21]. Studies by Aka et al. [22] showed the antidiabetic activity of aqueous extract (AE) and methanol extracts and fractions of *G. latifolium* in alloxan-induced diabetic rats.

Emphatically, medicinal plants including *Ocimum gratissimum, Gnetum africanum, Gongronema latifolium, Vernonia amygdalina,* and *Aloe Barbadensis* were selected for the preparation of poly-herbal formulation, used to investigate their hepaprotective efficacy in alloxan-induced diabetic rats.

2. MATERIALS AND METHODS

Materials

2.1 COLLECTION AND PREPARATION OF PLANT MATERIALS

The leaves of Vernonia amygdalina (VA), Gongronema latifolium (GL), Ocimum gratissimum (OG), Gnetum Africanum (GA)and Aloe barbadensis (AB) were purchased from a local market, Oriugba market in Umuahia Abia state, Nigeria and were authenticated by a Taxonomist (Dr Ibe K. Ndukwe) from the forestry department, College of Natural Resources and Environment Management (CNREM), Michael Okpara University of Agriculture Umudike.The leaves were sorted and peeled, washed with distilled water and then air-dried (at a temperature of 27 ^{o} C) to a constant weight. After one week, the leaves were milled into a powdered form using a mechanical homogenizer. The powders of the different plants were stored in clean containers and labelled accordingly for further use

Methods

2.2 PREPARATION OF PHF5

The powders of the different plants VA, GL, OG, AS and AB were mixed in the ratio of 3:3:3: 2:1 respectively to derive the poly-herbal formula (PHF) used for this study. The PHF was dissolved in hot distilled water and filtered after 2 minutes. The filtrate was freshly prepared for each administration and

was used to treat the animals in this study. The aqueous extracts of leaf were prepared according to the procedure of Dinesh et al**.** [23] and Padmanabhan et al. [24].

2.3 EXPERIMENTAL ANIMALS

Thirty (30) male Wistar rats (80-120g) were purchased from animal farm at the University of Nigeria Nsukka and used for this study. The animals were housed in aluminium cages (5 animals per cage) in clean conditions at an ambient temperature of 25^{o}C with a 12-hour light /dark cycle. They were fed standard feed and water *ad libitum*. The animals were acclimatized for two weeks before the commencement of the experiment. The Principles of Laboratory Animal Care (NIH, 1985) were followed throughout this study. All experimental procedures were conducted according to the animal ethics committee. The induction of diabetes was done intraperitoneally using alloxan monohydrate at the dose level of 100 mg/kg bodyweight

2.4 INDUCTION OF DIABETES

Alloxan monohydrate was dissolved in normal saline and administered to the animals at a dose level of 100mg/kg intraperitoneally to induce Type 2 diabetes. The animals were evaluated for fasting blood glucose levels in 72 hours after administration of the drugs to confirm induction. A fasting blood glucose level of 140 mg/dl was the criterion for selecting diabetic rats(Weir *et al.,* 1981).

2.5 DOSE SELECTION

The PHF was administered to the animals daily for 8 weeks. The doses were calculated for humans and modified for rats using the method of Paget and Barnes [25].

2.6 EXPERIMENTAL DESIGN

A total of 30 rats were used, and they were divided randomly into 6 different groups containing 5 animals per cage and treated as follows:

Group 1 – Normal control.

Group 2 – 100mg/kg bw of Alloxan + low dose (75 mg/kg bw) of PHF5

Group 3 – 100mg/kg bw of Alloxan + medium dose (150 mg/kg bw) of PHF5

Group 4 – 100mg/kg bw of Alloxan + high dose (300 mg/kg bw) of PHF5

Group 5 – 100mg /kg bw of Alloxan only

Group 6 – Glibenclamide (Glanil) (5 mg/kg bw) + 100mg /kg bw Alloxan

The administration of PHF5 was done orally, and the fasting blood glucose level was checked weekly using a glucometer during the treatment period by collecting blood from the tail vein of the animals. Bodyweight variations were monitored weekly throughout the period of the experiment.

2.7 ACUTE ORAL TOXICITY

The acute oral toxicity of the poly-herbal formulation was carried out following the Organization for Economic Cooperation and Development (OECD) guidelines. Three animals per dose were used for the experiment. Overnight fasted rats were orally fed with PHF5 in dose levels of 200, 400, 800, and 1000 mg/kg bodyweight , respectively. The animals were continuously observed for their behavioural (alertness, restlessness and irritability), touch response, pain response and spontaneous activity, and autonomic (defecation and urination) profiles for 24 h. After 24 h, the animals were observed for 14 days for mortality.

2.8 COLLECTION AND PREPARATION OF SAMPLES

At the end of the administration period, the animals were anaesthetized, and blood samples were collected via cardiac puncture. The blood samples were stored in clean vacutainer tubes and centrifuged at 4000 g for 15 minutes. The serum was used for the estimation of biochemical markers such as Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) using Randox Diagnostic kits.

2.9 DETERMINATION OF BIOCHEMICAL PARAMETERS

2.9.1 Assay of serum alanine aminotransferase (ALT) activity

Principle: Serum ALT activity was estimated by Reitman and Frankel [26] as outlined in Randox KIT. The method is based on pyruvate production by the transamination activity of ALT reacted with 2.4-ditrophenylhydrazine (DNPH), which gives a brown-coloured hydrazone that can be measured colourimetrically at wavelength 550 nm.

α-Oxoglutarate + L-alanine $\xleftrightarrow{\text{ALT}}$ L-glutamate + Pyruvate

2.9.2 Assay of serum aspartate aminotransferase (AST) activity

Principle: Serum AST activity vas estimated according to the method of Reitman and Frankel [26]. as outlined in Randox Kit. Oxaloacetate reacts with AST, which decarboxylates it spontaneously to pyruvate

measured by hydrazone formation after pyruvate reacts with 2, 4-dinitrophenyl hydrazine (DNPH), which gives a brown-coloured hydrazone that can be measured colourimetrically at 510nm.

α-Oxoglutarate + L-alanine $\xleftrightarrow{\text{AST}}$ L-glutamate + oxaloacetate

2.9.3 Assay of serum alkaline phosphatase (ALP) activity

The activity of alkaline phosphatase (ALP) was assayed using the method of Kochmar and Moss [27].

Principle: In the presence of magnesium and zinc ions, p-nitrophenol phosphate is hydrolyzed by phosphatase to form phosphate and p-nitrophenol. The p-nitrophenol released is proportional to the alkaline phosphatase (ALP) activity and can be measured photometrically.

P-nitrophenylphosphate + H_20 $\xrightarrow{\text{Alkaline Phosphatase}}$ Phosphate + P-nitrophenol

2.9.4 Serum total protein

Total protein estimation was assayed using the direct Biuret method [28].

Principle: This method's principle is that serum proteins react with copper sulphate in sodium hydroxide to form a violet complex called the Biuret complex. The intensity of the violet colour is proportional to the concentration of the protein.

2.10 STATISTICAL ANALYSIS

Data obtained were expressed as mean ± SD and statistically analyzed using one-way analysis of variance (ANOVA) with Turkey's multiple comparison post hoc tests to compare the level of significance between the test groups. The values of $p<0.05$ were considered significant.

3. RESULT

3.1 Toxicity study of PHF5

Acute toxicity studies showed no mortality up to 2000 mg/kg given as single oral administration mg/kg. The study was done at three different dose levels (75, 150, and 300 mg/kg).

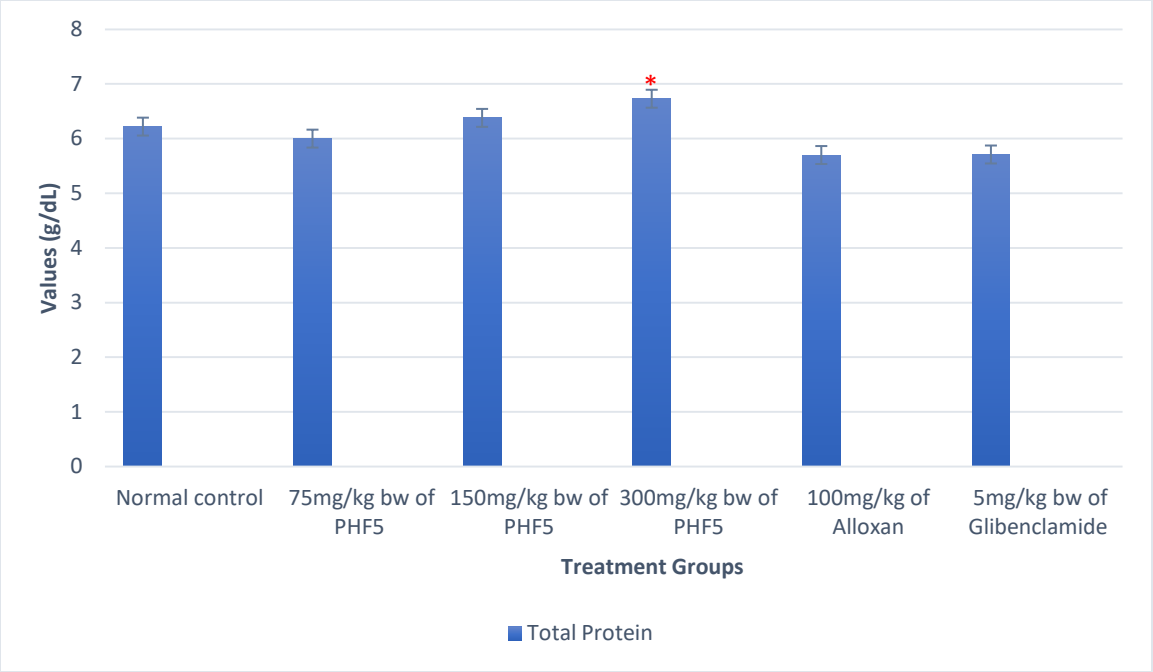

Fig.1 Effect of PHF5 Extract on Total protein

PHF5: Polyherbal formula. Values are expressed as mean ± SEM (n=6). *$p<0.05$ when compared with the negative control TP: Total Protein.

The results of the total protein activities of the studied rats are presented in Fig.1. As shown in the Figure, there was a statistically significant ($p<0.05$) decrease in the total protein of the diabetic control group when compared to the normal control. However, at 300 mg/kg, the PHF5 was able to significantly ($p<0.05$) increase total protein in the animals compared to normal control.

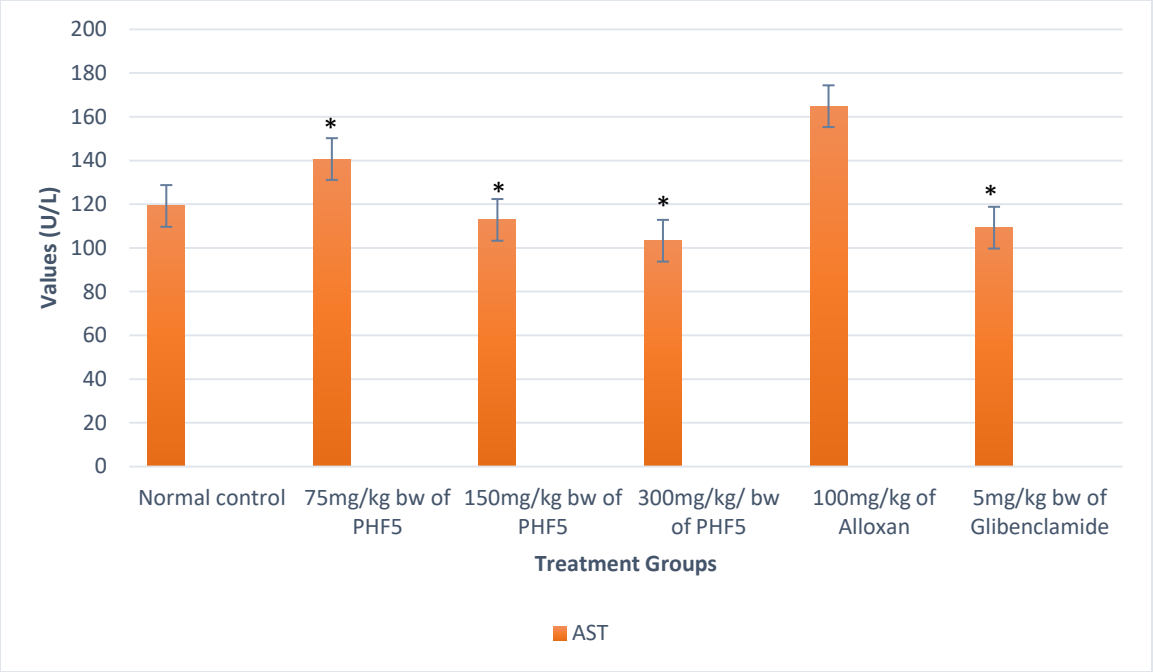

Fig. 2. Effect of PHF5 Extract on Aspartate Amino Transferase (AST) serum enzymes

PHF5: Polyherbal formula. Values are expressed as mean±SEM (n=6). *$p<0.05$ when compared with the negative control. AST: Aspartate Amino Transferase

Data for the AST activities were significantly ($p<0.05$) increased in the diabetic control animals compared to the normal animals. At 75 mg/ kg, 150 mg/kg and 300 mg/kg, the PHF5 significantly decreased AST compared to the normal control.

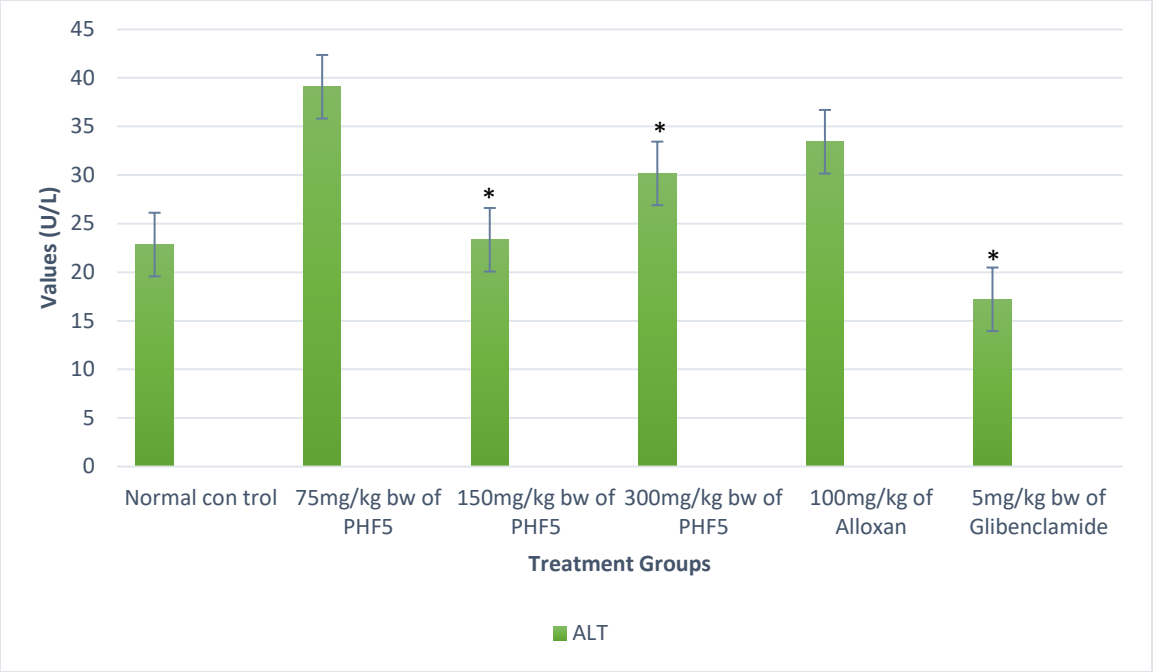

Fig. 3. Effect of PHF5 Extract on Alanine Amino Transferase (ALT) serum enzymes

PHF5: Polyherbal formula. Values are expressed as mean ± SEM (n=6). *$p<0.05$ when compared with the negative control. ALT: Alanine Amino Transferase

Data for the ALT enzyme activities were significantly ($p<0.05$) increased in the diabetic control animals compared to the normal animals. At 150 mg/kg and 300 mg/kg, the PHF5 significantly decreased AST compared to the normal control.

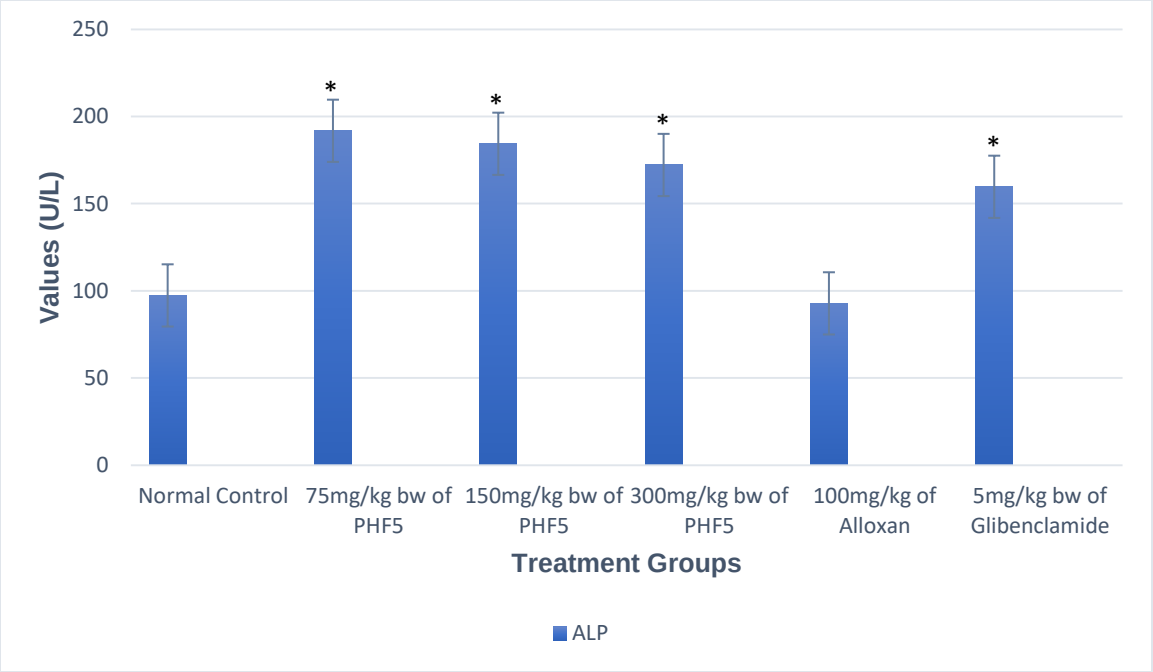

Fig. 3. Effect of PHF5 Extract on Alkaline Phosphatase (ALP) serum enzymes
PHF5: Polyherbal formula. Values are expressed as mean ± SEM (n=6). *$p<0.05$ when compared with the negative control. ALP: Alkaline Phosphatase

Data for the ALP enzyme activities were significantly ($p<0.05$) reduced in the diabetic control animals compared to the normal animals. Supplementation of poly-herbal formula at 150 mg/kg and 300 mg/kg significantly elevated ALP activity compared to the diabetic control animals. The Glibenclamide-treated animals also showed significantly higher ALP activity than the diabetic control.

DISCUSSION

Herbal formulations have gained much attention in treating diseases, owing to their efficacy with minor known side effects and easy access [29]. According to studies, polyphenolic compounds effectively prevent the development of long-term diabetes and its complications [30]. This present study investigated the hepaprotective action of *Ocimum gratissimum, Gnetum africanum, Gongronema latifolium, Vernonia amygdalina* and *Aloe Barbadensis* leaf extracts and their effects on biochemical parameters of induced alloxan diabetic wister rats.

In the present study, hyperglycemia caused by alloxan resulted in a significant ($p<0.05$) decrease in total protein, ALP and an increase in AST and ALT plasma levels. The decrease in total protein level could indicate a decrease in the rate of protein synthesis or an increase in protein breakdown, following the

previous studies by Suriawinata and Thung [31]. Recently, decreased protein synthesis has been accord for microproteinuria, which has been accounted to precede the development of overt nephropathy in diabetes mellitus [32]. However, at the dose level of 300 mg/kg of PHF5 extracts increase the serum total protein level which may be safe dose level for the animals.

The levels of alanine transaminase, aspartate transaminase, and alkaline phosphatase are essential indicators for assessing the severity of the injury. The effects of hyperglycaemia-induced oxidative stress on the liver are primary organs susceptible to it [33]. The increase in activities of these enzymes can be attributed to alloxan toxicity which leads to liver damage. However, the dose-dependent (75 mg/ kg, 150 mg/kg and 300 mg/kg) treatment with poly-herbal formula (PHF5) for eight weeks was able to attenuate the damage caused to the liver as evidenced by the reduced enzyme activity in the animals. This result was in agreement with the previous of Thapa and Anuj [34], who reported that ALT (10 – 55 µ/L), AST (10 – 40 µ/L), and ALP (45 – 115 µ/L) are the standard range of accepted values for liver function tests, beyond which liver disease can be suspected. From our findings, liver function parameters remained within normal range after administering a polyherbal formula (PHF5) in all the treated groups, suggesting the hepatoprotective properties of the alloxan-induced Wistar rats, which is in tandem with Thapa and Anuj [34].

CONCLUSION

Based on the result obtained from this study, it is concluded that the best dose level of PHF5 extracts that yield the best result with good efficacy and less hepatotoxicity on biomarker enzymes is 300 mg/kg and it is therefore recommended for synthesizing a standard anti diabetic drugs if further purified.

ETHICAL APPROVAL

The study was conducted following the National Institute of Health guidelines, the USA, as approved by the College of veterinary medicine, Michael Okpara University of Agriculture, Umudike. The ethical committee's reference number is: MOUAU/CVM/REC/202015

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