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EVALUATION OF LIVER MARKER ENZYMES AND BIOCHEMICAL INDICES OF ALLOXAN INDUCED DIABETIC WISTAR RATS TREATED WITH AQUEOUS EXTRACT OF *Pennisetum purpureum*

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ABSTRACT

Liver marker enzymes, total protein, amylase and glucose were evaluated in alloxan-induced diabetic wistar rats treated with aqueous extract of Pennisetum purpureum. The liver marker enzymes evaluated were alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Sixteen wistar rats were grouped into four which were administered with alloxan; a toxic glucose analogue that caused a hyperglycaemic state. 12 and 15% aqueous extract of P. purpureum were administered to these rats at a dose of 20mg/kg. The result of this study showed that there was significant elevation (P<0.05) of glucose, AST, ALT, total protein (TP) and amylase in comparison to groups 1 and 2. The values of AST, ALT and glucose in groups 3 and 4 significantly decreased in comparison to groups 2, while the total protein and amylase showed no significant change. The aqueous extract of P. purpureum significantly lowered the blood glucose, AST and ALT. Total protein and amylase were not significantly lowered when administered with the 12% of extract but were significantly lowered when administered 15% of the extract. This study reveals that the aqueous of P. purpureum significantly lowered the biochemical parameters studied on alloxan-induced diabetic wistar rats by significantly (P<0.05) lowering blood glucose, AST and ALT and amylase levels and an elevation in the Total protein level, hence pointing in the direction of the use of this plant as an anti-diabetic agent in alloxan-induced diabetic rats.

Key words: Toxic glucose analogue, Therapeutic target, Hyperglycaemicstate.

INTRODUCTION

Pennisetum purpureum (Napier) is tall and robust bamboo-like clumps (Farrel*et al.,* 2002). It requires low water and nutrient inputs to grow (Strezov*et al.,* 2008). The protein content of *P. purpureum* is higher than those reported for *Nypa fruiticans* fruits and seeds (Osabor*et al.,* 2008), *Boerhavia diffusa* and *Comelina nudiflora* (Ujowundu *et al.,* 2008), *Trichosanthes anguina* (Ojiako and Igwe, 2008) and compares with that of *Parkia biglobosa* (Esenwah and Ikenebomeh, 2008). The ash value of *P. purpureum* is relatively high compared with reported values for meat and egg, and comparable with that of wheat flour (Singh, 2004).

Diabetes mellitus is a disease widespread with a high degree affecting persons both in developed and developing countries. The World Health Organization (WHO)

estimated that more than 220 million people around the world suffer from diabetes, with 80% occurring in low and middle income countries. Experts expect this number to increase to more than 400 million by the year 2030 (Wild, 2004).

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger) (Cooke and Plotnick, 2008).

Alloxan-induced diabetes has been commonly employed as an experimental model of insulin dependent diabetes mellitus (IDDM). The mechanism of alloxan action has been thoroughly studied which currently can be characterized quite well. Several experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after alloxan treatment (Lachin and Reza, 2012).

This study was designed to evaluate liver marker enzymes, total protein and amylase in alloxan induced diabetic wastar rats treated with aqueous extract of *P. purpureum.*

MATERIALS AND METHODS

Preparation of Plant Extract

Pennisetum purpureum (Elephant grass), used in this experiment, was obtained from Abuja Campus, University of Port Harcourt, Nigeria. The leaves were carefully plucked, healthy fresh leaves were sorted, thoroughly washed without squeezing and dried under room temperature for four days. The dried leaves were ground into fine powdery form with pestle and mortar. The leaves were weighed before and after drying. 50g of the dried leaves powder was soaked in 500ml of distilled water. After vigorous shaking for 5 minutes, the mixture was allowed to stand for 24hrs before filtering with a clean filter paper. The filtrate was stored in the refrigerator and then 14mg/ml and 18mg/ml dilution were prepared from stock as reported by Omeodu*et al.*, (2008).

Experimental Animals

A total of twenty (20) healthy wistar albino rats of both sexes, weighing 130 – 290g were used for this experiment. They were obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka, Enugu State and transported to the Department of Biochemistry, University of Port Harcourt animal house. They were housed in stainless street cages (5 rats per cage) and kept in a well-ventilated room, in an animal laboratory under standard conditions. The rats were fed with standard diet (Livestock Feed Nig. Ltd, Ikeja, Nigeria) and water*ad libitum.* The animals were kept for one (1) week to acclimatize prior to the commencement of the experiment. The standard guidelines for the use of experimental animals (including applying humane actions during sacrifice) were adhered to.

Preparation and Induction of Alloxan

1.5g of alloxan was dissolved in 100ml of normal saline. Diabetes was induced by a single injection of freshly prepared alloxan monohydrate (120mg/kg). It was administered intraperitoneally (IP) to rats after fasting for 12 hours. Blood glucose level was measured four (4) days after alloxan administration using a glucose metre.

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Effect of Extracts

The rats were divided into four groups, each consisting of five rats, on the basis of their weights. The five rats in group 1 (normal control) were fed with rat feed and water only throughout the experimental period. Parameters from this group served as baseline data. The rats in group 2 (positive control) were maintained on the same feed as the normal control but in addition, they were diabetic.

The rats in group 3 (test 1) were maintained on the same diet as group 1 and 2. They were also diabetic but were treated with 14mg of the extract at a dose of 20mg/kg throughout the period of the research. The rats in group 4 (test 2) were also diabetic and were maintained on the same diet as other groups but were treated with 18mg of the extract at a dose of 20mg/kg.

For analysis, the rats from each group were sacrificed after 7 days of the treatment, and after they fasted for about 12 hours. They were made unconscious after anesthetics with chloroform before sacrificing them slitting their throat and allowing free flow of fresh blood into sterile bottles to obtain serum. The blood samples were then analyzed in the Chemical Pathology Laboratory of the University of Port Harcourt Teaching Hospital.

Enzyme Assays

The determination of aspartate aminotransferase in the serum samples were performed at 37°C using the Randox Kit by measuring the amount of oxalracetatehydrazone formed in the presence of L-aspartate, α-oxoglutarate and 2,4-dinitrophenyl hydrazine as reported by Ibekwe*et al.,* (2007). For alanine aminotransferase, L-alanine replaced L-aspartate and peptide bonds of protein with biuret reagent in an alkaline medium to give a purple colour. The intensity of the colour which is measured at 540nm is proportional to the protein concentration. The determination of glucose occurs after enzymatic oxidation in the presence of glucose oxidase. The hydrogen perioxide formed reacts under catalysis of perioxidase, with phenol and 4-aminophenazone to form a red violet quinine imine dye as indicator. α-amylase is present as a catalyst in the breakdown of Benzylidiene G7PNP to yield Benz-G and PNP-oligosaccharide while glucoamylase serves as a catalyst in the degradation of PNP-Oligosaccharide to yield Glucose and PNP-Glucoside.

Statistical Analysis

All data were statistically analyzed using statistical package of Microsoft Excel 2007 Window 7 starters. One way ANOVA was used and P value were considered significant at $P < 0.05$.

RESULTS

The results were represented in figures, in form of bar charts.

The result of this study showed that there were significant differences of glucose, AST, ALT, total protein and amylase in the comparison of group 1 and group 2 and group 4; and also group 1 and group 3 at $P < 0.05$.

The value of AST, ALT and glucose in the comparison of group 1 and group 2 and group 2 and group 3 were significantly different at $P < 0.05$ while the total protein and amylase had no significant difference as P – Value is > 0.05. Also, the glucose, AST, ALT, total protein and amylase in the comparison of group 4 and 3 is not significant at $P > 0.05$.

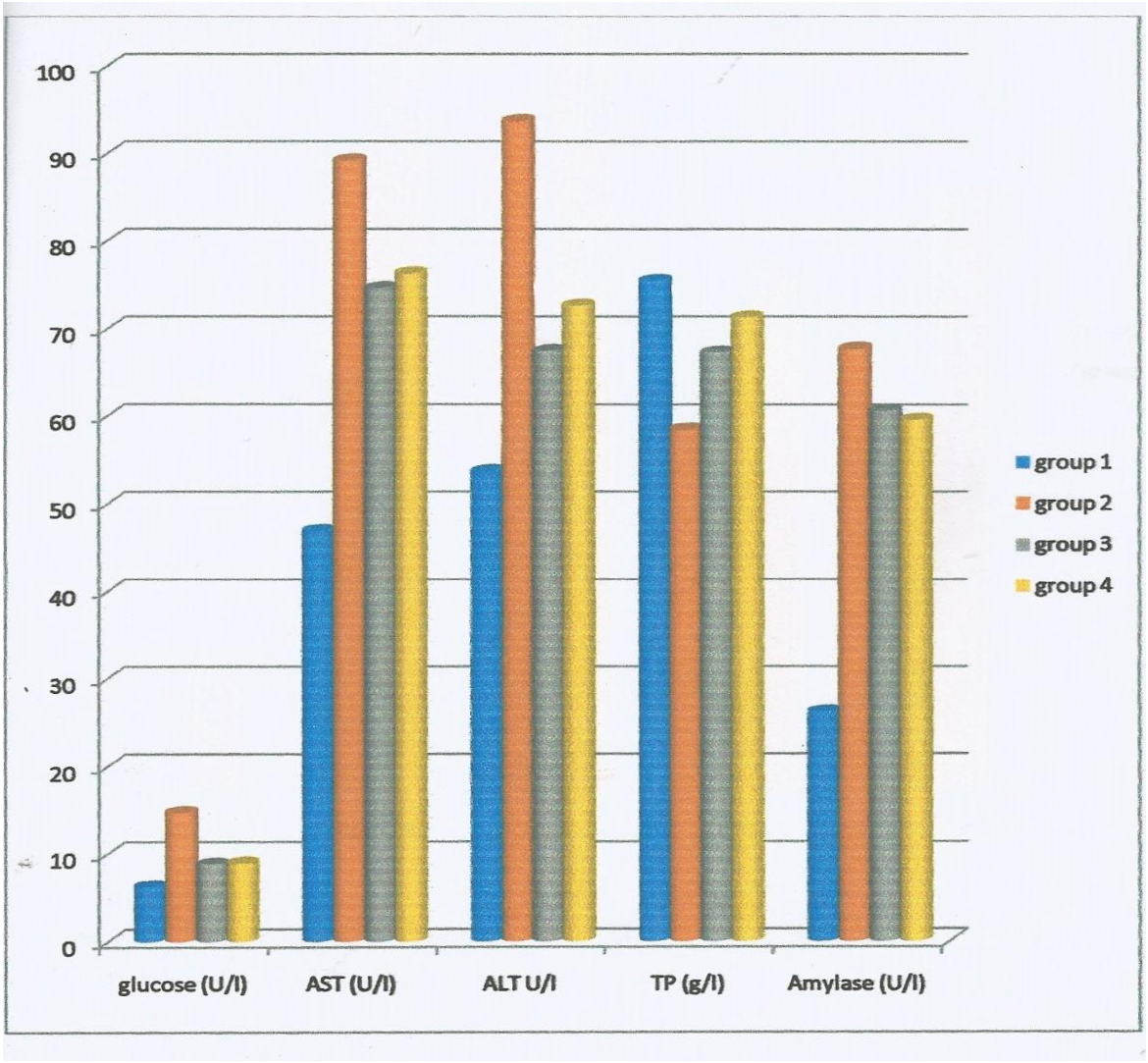

Figure 1: Comparison of glucose, AST, ALT, total protein and amylase levels in group 1, group 2, group 3 and group 4.

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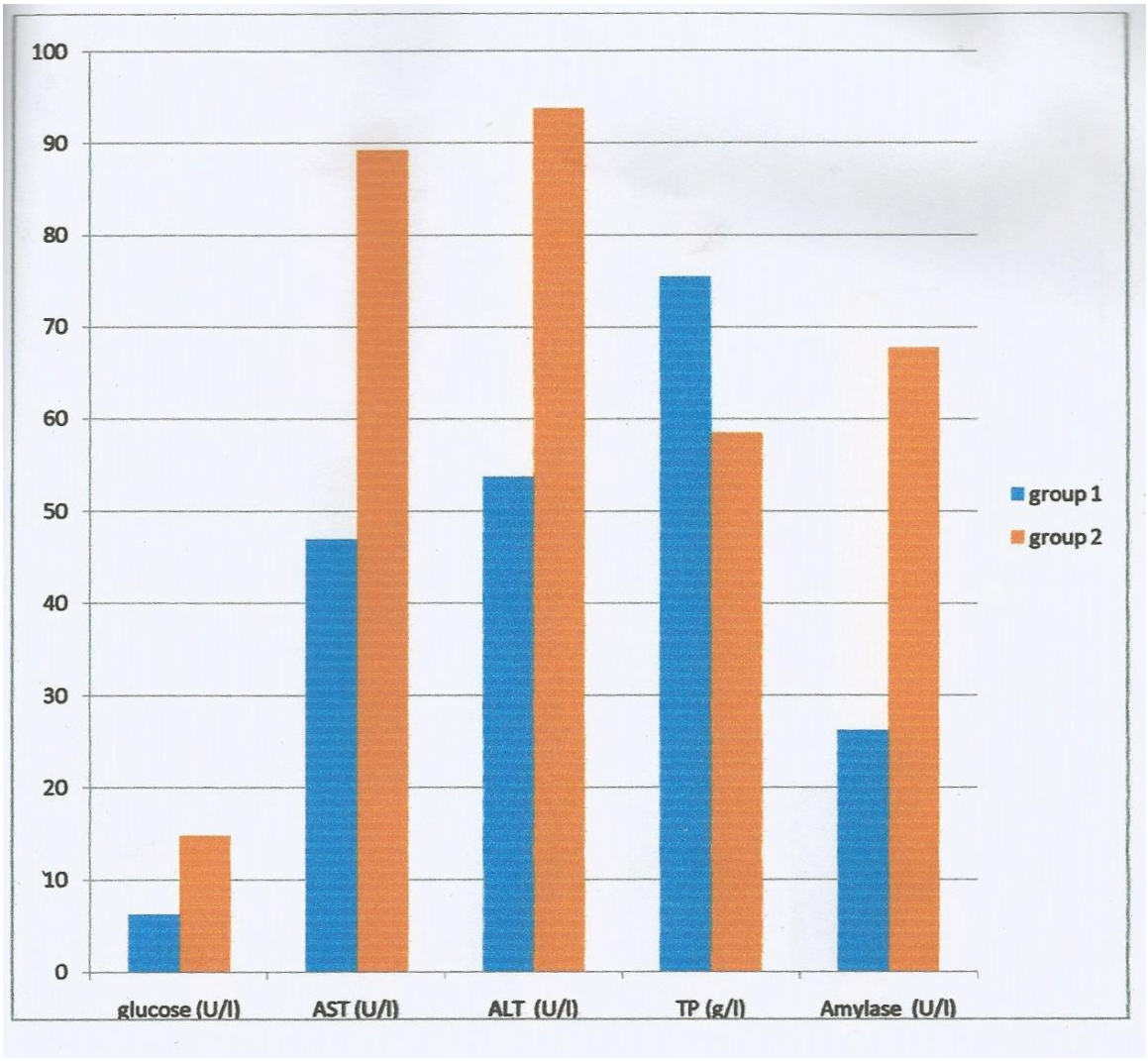

Figure 2: Comparison of glucose, AST, ALT, total protein and amylase levels in group land group 2.

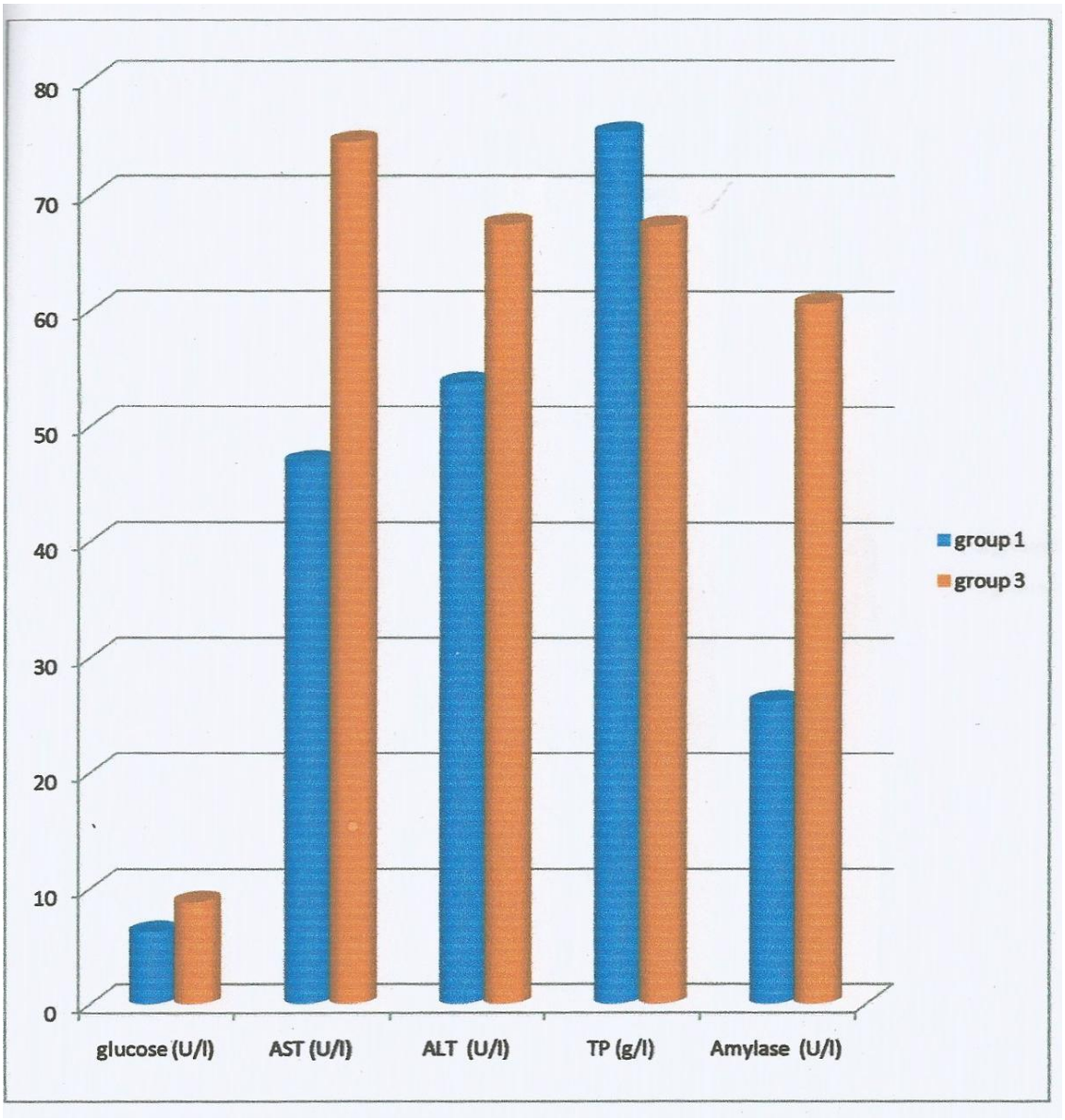

Figure 3: Comparison of glucose, AST, ALT, total protein and amylase levels in group 1 and group 3.

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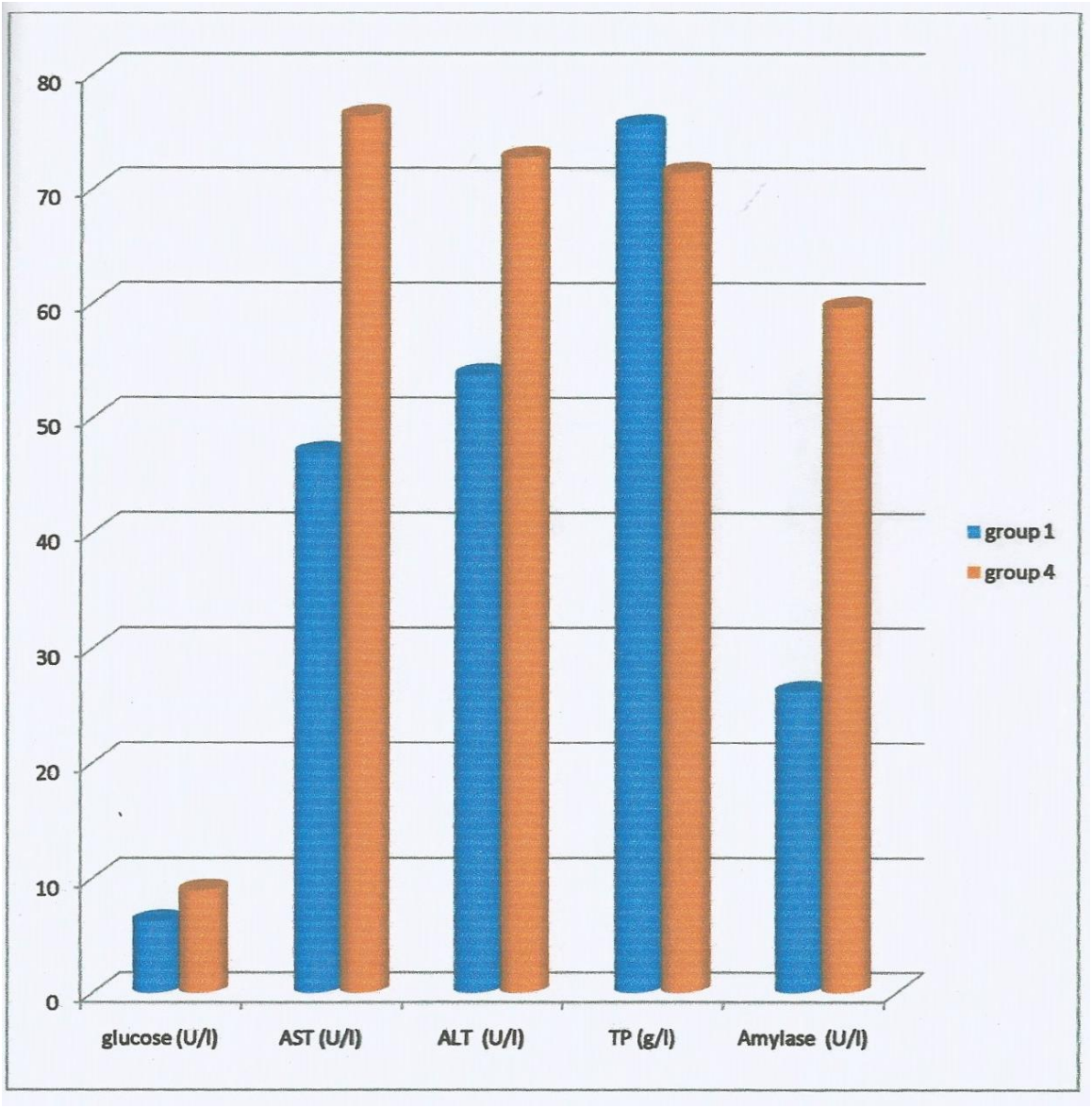

Figure 4: Comparison of glucose, AST, ALT, total protein and amylase levels in group 1 and group 4.

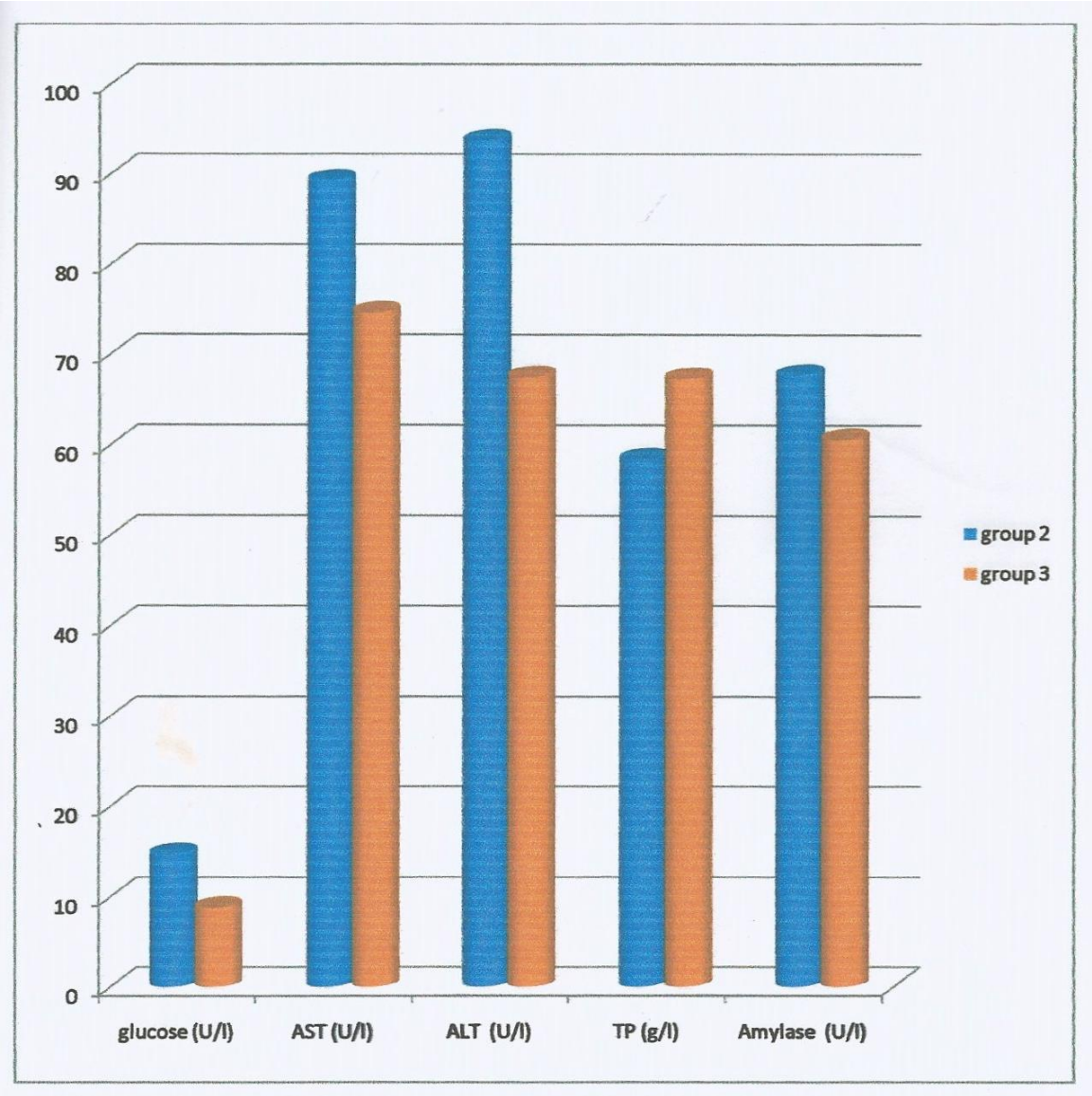

Figure 5: Comparison of glucose, AST, ALT, total protein and amylase levels in group 2 and group 3.

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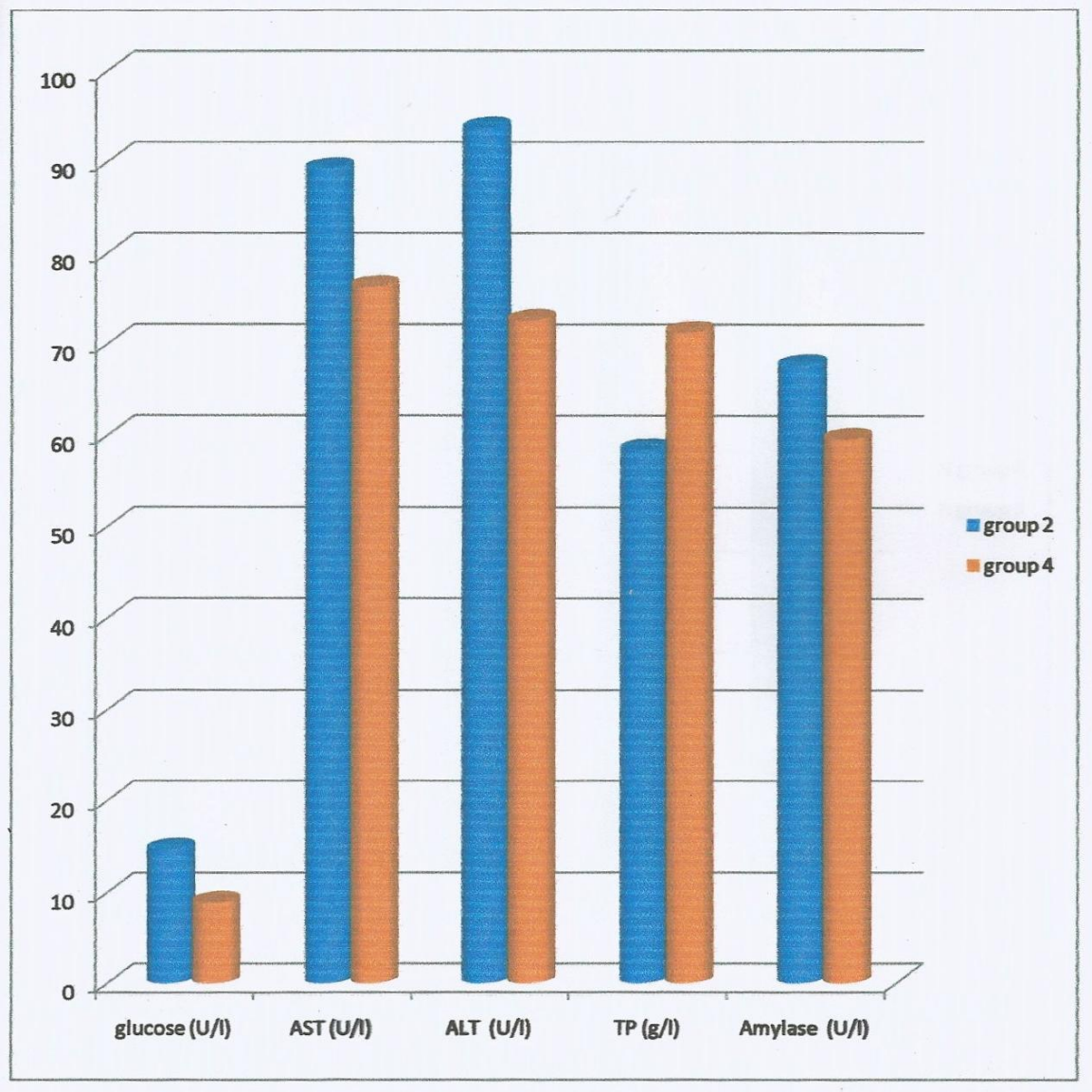

Figure 6: Comparison of glucose, AST, ALT, total protein and amylase levels in group 2 and group 4.

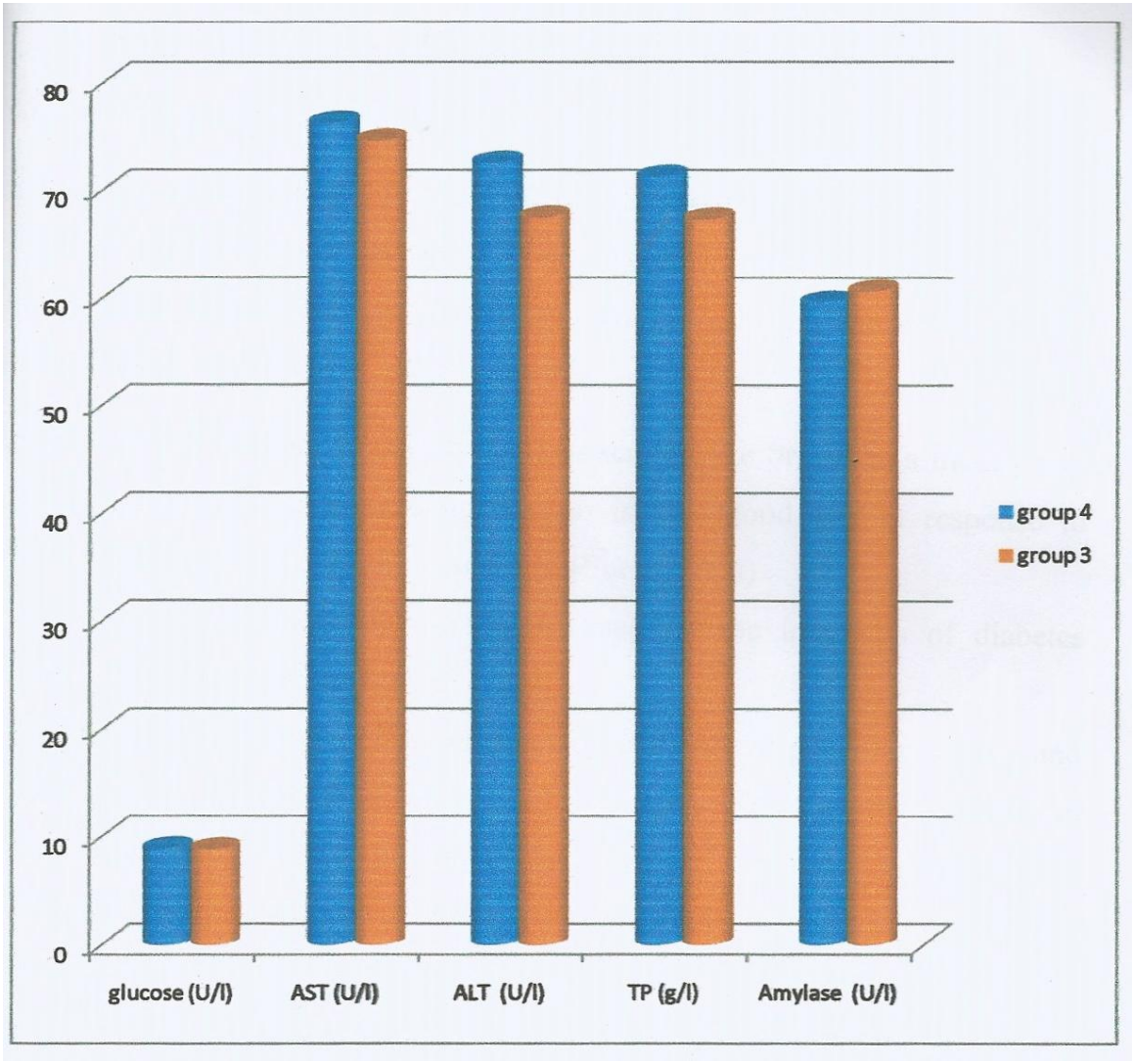

Figure 7: Comparison of glucose, AST, ALT, total protein and amylase levels in group 4 and group 3.

DISCUSSION

Alloxan is one of the usual substances used for the induction of diabetes mellitus apart from streptozotocin. Insulin deficiency leads to various metabolic alterations in the animals via increased blood glucose, increased levels of transaminases etc.

This study showed that the aqueous extract of *P. purpureum* had a significant effect on glucose, AST and ALT of the Alloxan induced diabetic rats when administered 14 mg and 18 mg at a dose of 20mg/kg (Omeodu et al., 2008). Total protein and amylase had no significant effect when administered 14 mg of the extract but had a significant effect when administered 18 mg of the extract at a dose of 20mg/kg on the diabetic rats.(Omeodu et al., 2008). Further pharmacological and biochemical investigations will clearly elucidate the mechanism of action and will clearly elucidate the mechanism of action and will be helpful in projecting this plant as a therapeutic target in diabetes research. In this research, just ALT and AST liver

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marker enzymes were considered, other liver marker enzymes such as GGT and ALP should be included in further research.

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