



EFFECT OF *PISUM SATIVUM* SEEDS SUPPLEMENT ON BLOOD GLUCOSE LEVEL AND SOME BIOCHEMICAL PARAMETERS IN ALLOXAN-INDUCED DIABETIC WISTAR RATS

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ABSTRACT

Diabetes is the fastest growing chronic disease which has become a major concern all over the world. The study was carried out to investigate the effect of *Pisum sativum* seed supplement on blood glucose level, some biochemical parameters and histological studies in Alloxan-induced diabetic Wistar rats. Alloxan monohydrate was used to induce hyperglycaemia (blood glucose level ≥ 180 g/dL) in twenty-five rats which were divided into five groups of five each. Group 1: received 1m/kg of normal saline and fed with rats feed, group 2: received 1mg/kg of glibenclamide orally in addition to normal feed daily, while groups 3, 4 and 5 were fed with 5%, 10% and 20% of *P. sativum* seed supplement with normal feed for fourteen days. The Blood glucose level of the rats was determined at intervals of three days during the study period. At the end of the study period, the rats were sacrificed and their pancreas harvested for histopathology. Blood was collected and plasma samples were used for the evaluation of lipid profile and liver enzymes. The results obtained in this study showed *P. sativum* seed supplement decreased blood glucose level with more effectiveness than the standard drugs, non-significant change was seen in serum lipids and ALP with increased serum concentrations of AST and ALT in Alloxan-induced diabetic rats.

Keywords: Alloxan, Glibenclimide, Liver enzymes, Lipid profiles, *Pisum sativum*

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism which results from defects in insulin secretion, insulin action, or both [1]. Chronic hyperglycaemia can cause pathological and functional changes for a long time before the diagnosis of diabetes is made [2]. Diabetes mellitus if untreated or not diagnosed correctly could lead to long term damage, dysfunction and failure of various organs [3]. There are various forms of diabetes mellitus which come about due to different causes. Diabetes may be primary, in the sense that, it is not related to another disease or secondary diabetes that occurs as a result of damage or injury to the pancreas caused by disease. Primary diabetes is divided into Type 1, Type 2, gestational and other forms of diabetes [1]. Diabetes is one of the oldest diseases that date back since 1425 [4].

The incidence of diabetes mellitus is increasing rapidly. About 285 million people had diabetes in 2010 and raise to 382 million affected people in 2013, of which 80% were affected with Type 2 diabetes mellitus [5]. About 79, 000 children developed Type1 diabetes mellitus, more than 21 million live births were affected by gestational diabetes and 175million of the people

affected were undiagnosed and it causes about 5.1 million deaths in 2013 [6].

Chronic hyperglycaemia leads to inadequate supply of glucose to the brain, resulting in impairment of neural function, causing seizures, unconsciousness and permanent brain damage or death [7]. Diabetes mellitus is therefore a weakening disease with life-threatening complications and the burden associated with the management of this disease is vast [8]. (*Pisum sativum* (*P. sativum*) is a leguminous vegetable which belongs to the family Fabaceae, which is rich in phyto-nutrients, minerals, vitamins and anti-oxidants that are of health benefit [9]. In this context an attempt was made in the present study to investigate the anti-diabetic potentials of the *Pisum sativum* seeds on blood glucose, lipid profile and liver enzymes in Alloxan-induced diabetic Wistar rats with respect to phytochemicals.

MATERIALS AND METHODS

Plant material

Fresh *Pisum sativum* seeds were purchased on August, 2014 from a farm in Saminaka, Lere Local Government, Kaduna State, Nigeria. Authentication and identification were carried out by Malam Namadi Sunusi of Department of Botany, Ahmadu Bello University, Zaria.

A specimen was deposited and a voucher number 1798 was assigned for future reference.

Experimental animals

A total of twenty-five adult Wistar rats of both sexes weighing 150-180 grams were used for this study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. They were allowed access to food and water *ad libitum* throughout the period of the experiment except when the animals were required to be fasted according to experimental protocol (Permission for approved use of animal by Animal Ethical Use of the University).

Drugs and chemicals used

Chemicals used include: Chloroform (606049 BDH Chemicals Pole, England), formalin (10%), methylated spirit (3025 BDH Chemicals Pole England), and normal saline, glibenclamide (AF1503, McW Health (P) LTD, Sector-E, Industrial Arch, Sanwer Road, Indore (M.P.) India) and Alloxan monohydrate (Sigma-Aldrich chemical Company, St. Louis U.S.A.).

Equipment and consumables

Equipment and consumable reagents used for the study includes: Digital Glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany), Weighing Balance (GF 2000 Germany), Centrifuge (Denley BS400, England), , India), Automatic tissue processor (Shandon, Southern Duplex LTD 1319, England), sledge microtome (JB-4 Searchtech LTD, England), Dissecting set, Petri-dish, plain bottles, cotton wool, syringes, hand gloves, bowls, drinkers, pins and needles.

Plant preparation

The plant seed was shade dried to a constant weight and then grounded to powder. The powder was thereafter added to the animal feed in percentage proportions. The feed supplements were prepared by mixing 5 g of the powdered *P. sativum* with 95 g of normal rat feed to make 5% of the *P. sativum* supplement. The 10% and 20% of the *P. sativum* supplement was prepared by mixing 10 g and 20 g of powdered *P. sativum* with 90 g and 80 g of normal rat feed respectively [10].

Induction of diabetes mellitus

The method of Katsumata and co-workers [11] was used to induce diabetes. Rats that were fasted for 16-18 hours were given a single intraperitoneal (IP) injection of Alloxan monohydrate dissolved in 0.9% cold normal saline solution at a dose of 150 mg/kg body weight. These rats were then given 20% glucose solution orally after 6 hours of Alloxan monohydrate administration and were kept for the next 24 hours on 5% glucose solution in their cages to prevent hypoglycemia [12]. After 72 hours of Alloxan administration, blood was

collected from the tail vein of each rat for assessment of blood glucose level using a digital glucometer. Rats having fasting blood glucose level of 180 mg/dl and above were chosen for the study [12].

Experimental design

This study was conducted for fourteen days. The animals were randomly divided into five groups of five each after the diabetes induction as follows Group 1 (Negative control) Diabetic rats which were given 1ml of normal saline and fed with normal rat feed, Group 2 (Positive control) Diabetic rats that were given 1mg/kg body weight of glibenclamide orally per day and fed with normal rat feed, while Groups 3, 4 and 5 were fed with 5%, 10% and 20% of *P. sativum* supplement mixed in rat feed respectively.

Determination of blood glucose levels

Determination of blood glucose level was done by the glucose-oxidase principle [13]. Fasting blood glucose levels was determined before the induction of diabetes using a Digital glucometer and the results expressed in the unit of mg/dl. Blood samples were collected from the tail vein of the rats after confirmation of the diabetes at intervals of 0, 3, 6, 9, 12 and 14 days and the blood glucose levels were determined respectively.

Collection of samples

After the study period all animals were anesthetized using chloroform and thereafter sacrificed. Blood samples were obtained from each animal by cardiac puncture and the samples collected were kept in plain bottles. The serum of samples collected was thereafter used for biochemical analysis. The pancreas of each rat was harvested immediately and fixed in 10% formalin to prevent tissue autolysis.

Estimation of Serum Lipids

Determination of serum total cholesterol

The serum level of total cholesterol (TC) was quantified after each enzymatic hydrolysis and oxidation of the sample as described by Stein [14]. To 1 ml of the sample and the standard, 1000 µl of the reagent was added respectively. This was incubated for 10 minutes at 20-25°C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) were measured against the blank reagent within 30 minutes at 546 nm. The value of TC present in the serum was calculated using the following formula and expressed in mg/dl.

TC concentration = $A_{\text{sample}} / A_{\text{standard}} \times 196.86 \text{ mg/dl}$ [14].

Determination of serum triglyceride

The serum triglyceride (TGL) level was determined after enzymatic hydrolysis of the sample with lipases as described by Tietz [15]. To 0.5 ml of the sample and 0.5ml of the standard, 1000 µl of the reagent was added to each. This was incubated for 10 minutes at 20-25°C after mixing and the absorbance of the sample (A_{sample}) and standard (A_{standard}) was measured against the reagent blank within 30 minutes at 546 nm. The value of triglyceride present in the serum was calculated using the formula below and expressed mg/dL.

$$\text{TGL concentration} = A_{\text{sample}} / A_{\text{standard}} \times 194.0 \text{ mg/dL}$$
 [15]

Determination of serum high-density lipoprotein cholesterol

The serum HDL-C was measured by the method of Wacnic and Albers [16]. Low-density lipoproteins (LDL-C and VLDL-C) and chylomicron fractions in the sample was precipitated quantitatively by addition of phosphaturic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 x g. The supernatant represents the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined using the formula below and expressed in mg/dL

$$\text{HDL-C} = \text{TC} - (\text{LDL-C} + \text{VLDL-C} / \text{TGL})$$
 [16]

Determination of serum low-density lipoprotein cholesterol

The serum level of LDL-C was calculated using the TC, HDL-C and TGL values obtained according to the equation below:

$$\text{LDL-C} = \text{TC} - (\text{TGL}/5 + \text{HDL-C})$$

The value was expressed in mg/dL [17]

Estimation of Serum Liver Enzymes

The serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was determined, as follows:

Determination of serum aspartate aminotransferase (AST)

This was estimated by the method described by Bergmaya and Walefeld [18]. To 1000 µL of the samples, 100µl of the reagent was added and then mixed and incubated at 37°C for 1 minute. The change in absorbance of the sample (ΔAB) was measured per minute using a spectrophotometer at a wavelength of 590 nm.

$$\text{AST activity (U/L)} = \Delta AB / \text{min} \times 1768$$
 [18]

Determination of serum alanine aminotransferase (ALT)

This was estimated by the method described by Bergmaya and Walefeld [19]. To the samples 1000 µL of the reagent was added to 100µl of the samples, mixed and then incubated at 37°C for 1minute. The change in absorbance of the sample (ΔAB) was measured per minute using a spectrophotometer at wave length of 590 nm

$$\text{ALT activity (U/L)} = \Delta AB / \text{min} \times 1768$$
 [19]

Determination of serum alkaline phosphatase (ALP)

This was estimated by the method described by Bowers and Mc Comb [20]. To 0.5 ml of the sample 0.05 ml of the reagent was added, mixed and incubated at 37°C for 10 minutes. The change in absorbance of the samples was measured per minute using a spectrophotometer at wave length of 590 nm.

$$\text{ALP activity} = A_{\text{sample}} / A_{\text{standard}} \times \text{Value of standard (U/L)}$$
 [20]

Histological studies

Immediately after sacrifice, the rats were dissected. Pancreas of each rat was harvested and fixed immediately in 10% formol-saline for 48 hrs. The tissues were then processed in an automatic tissue processor and sections of 5µ thick were made using a sledge microtome and processed routinely by embedding in paraffin [21]. The sections obtained were stained in haematoxylin for 5minutes and thereafter placed in eosin for 1 minute. After the staining process, the sections were washed in tap water; air dried, cleared using xylene and mounted under a light microscope [22].

Statistical analysis

Data obtained from the present were expressed as mean \pm SEM. The difference between the groups were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett post hoc test to compare the level of significance between negative control and experimental groups. Values of $p \leq 0.05$ were considered as significant. Graph pad Prism statistical package was used for the analyses.

RESULTS

The effect of two weeks administration of *P. sativum* seed supplement in different percentages (5%, 10% and 20%) on blood glucose level shown in Table 1. Significant decrease in blood glucose level ($P < 0.05$) was seen in the treated groups when compared with the diabetic untreated group with even more potent antihyperglycaemic effect on the *P. sativum* treatment groups than in the positive control glibenclamide (1 mg/kg).

The non-significant decrease in the mean total cholesterol, triglycerides, high density lipoprotein cholesterol and low-density lipoprotein cholesterol within the treated groups when compared with the diabetic untreated group after two weeks *P. sativum* seed supplementation in different percentages (5%, 10% and 20%) on lipid profile (Table 2).

Table 3 shows effect of *Pisum sativum* supplement in different percentages (5%, 10% & 20%) on liver enzymes after two weeks. Significant increase ($P < 0.05$) in the serum concentration of ALT and AST in the diabetic rats fed with 20% of *P. sativum* supplement when compared with the diabetic untreated group.

Table 1: Effect of Two Weeks Administration of *Pisum sativum* Supplement on Blood Glucose Levels of Alloxan-induced Diabetic Wistar Rats

Blood Glucose Level (mg/dL)						
Groups	N	BGL0	BGL3	BGL6	BGL9	BGL12
DR NC	4	245.4 ± 8.4	309.6 ± 37.4	319.6 ± 36.8	323.4 ± 51.2	145.8 ± 62.9
DR PC	5	264.0 ± 11.0	215.8 ± 17.0	223.8 ± 20.1	210.8 ± 9.8	157.0 ± 21.7
DR 5%	5	245.8 ± 23.1	197.0 ± 4.1*	129.2 ± 10.2*	108.0 ± 8.2*	75.8 ± 5.9
DR 10%	5	248.8 ± 36.3	112.2 ± 29.7*	144.6 ± 25.9*	135.2 ± 28.1*	98.6 ± 15.5
DR 20%	4	248.6 ± 29.8	205.0 ± 24.7*	147.0 ± 26.7*	109.3 ± 16.9*	55.0 ± 22.5

Data expressed as Mean ± SEM, N: number of rats per group, DR NC: Diabetic rat negative control, DR PC: Diabetic rats treated with glibenclamide, Diabetic rats on 5%, 10% and 20% *P. Sativum* supplement

respectively; BGL: Blood glucose level, 0, 3, 6, 9, and 12: Days interval, * $P < 0.05$, the mean difference is statistically significant when compared with the negative control, Graphpad Prism statistical package.

Table 2: Effect of Two weeks Administration of *Pisum sativum* Supplement on Lipid profile of Alloxan-induced Diabetic Wistar Rats

GROUP	N	CHOL (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)
DR NC	3	139.6 ± 14.1	96.0 ± 26.2	66.3 ± 21.4	36.8 ± 7.3
DR PC	5	104.7 ± 6.3	115.6 ± 29.0	37.5 ± 2.2	44.1 ± 8.1
DR 5%	5	117.5 ± 8.4	100.1 ± 35.8	44.5 ± 5.7	53.0 ± 11.7
DR 10%	5	92.7 ± 10.6	45.9 ± 12.7	40.9 ± 16.0	31.0 ± 8.8
DR 20%	3	97.9 ± 27.2	45.5 ± 14.2	28.7 ± 3.6	59.3 ± 3.2

Data expressed as Mean ± SEM, N: number of rats per each group, DR NC: Diabetic rats negative control, DR PC: Diabetic rats treated with glibenclamide, Diabetic rats on 5%, 10% and 20% *P. sativum* supplements respectively, CHOL: Total Cholesterol, TG: Triglycerol, HDL-C: High density lipoproteins cholesterol, LDL-C: Low density lipoproteins cholesterol, * $P < 0.05$, the mean difference is statistically significant when compared with the negative control, Graph pad Prism statistical package.

Table 3: Effect of Two Weeks Administration of *Pisum sativum* supplement on Liver Enzymes of Alloxan-induced Diabetic Wistar Rats

Groups	N	ALT (Iu/l)	AST (Iu/l)	ALP (Iu/l)
DR NC	3	16.1 ± 7.2	19.5 ± 7.4	12.1 ± 4.0
DR PC	5	10.8 ± 4.8	16.8 ± 5.4	25.9 ± 11.5
DR 5%	5	13.9 ± 3.0	14.3 ± 3.2	23.3 ± 9.0
DR 10%	5	32.7 ± 21.3	19.5 ± 5.2	32.7 ± 21.3

DR 20%	3	38.1 ±10.9*	45.8 ±10.9*	22.4 ± 6.8
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Data expressed as Mean ± SEM, N: number of rats per each group, DR NC: Diabetic rats negative control, DR PC: Diabetic rats treated with glibenclamide, Diabetic Rats on 5%, 10% and 20% *P. Sativum* supplement respectively, ALT: Alanine Aminotransferase, AST: Aspartate Aminotransferase, ALP: Akaline phosphatase, I μ /L: International unit per litre, **P* < 0.05, the mean difference is statistically significant when compared with the negative control. Graph pad Prism statistical package

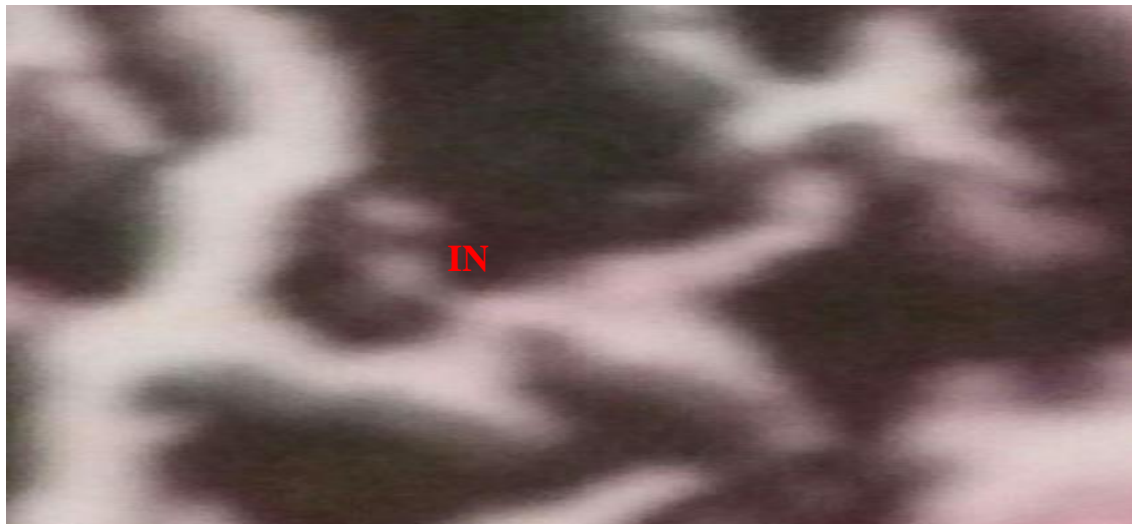


Plate I: Photomicrograph of pancreas from Alloxan-induced diabetic Wistar rats (Negative control) given 1 mL/kg of normal saline orally for two weeks showing islet cell necrosis (IN) (H and E x 250).

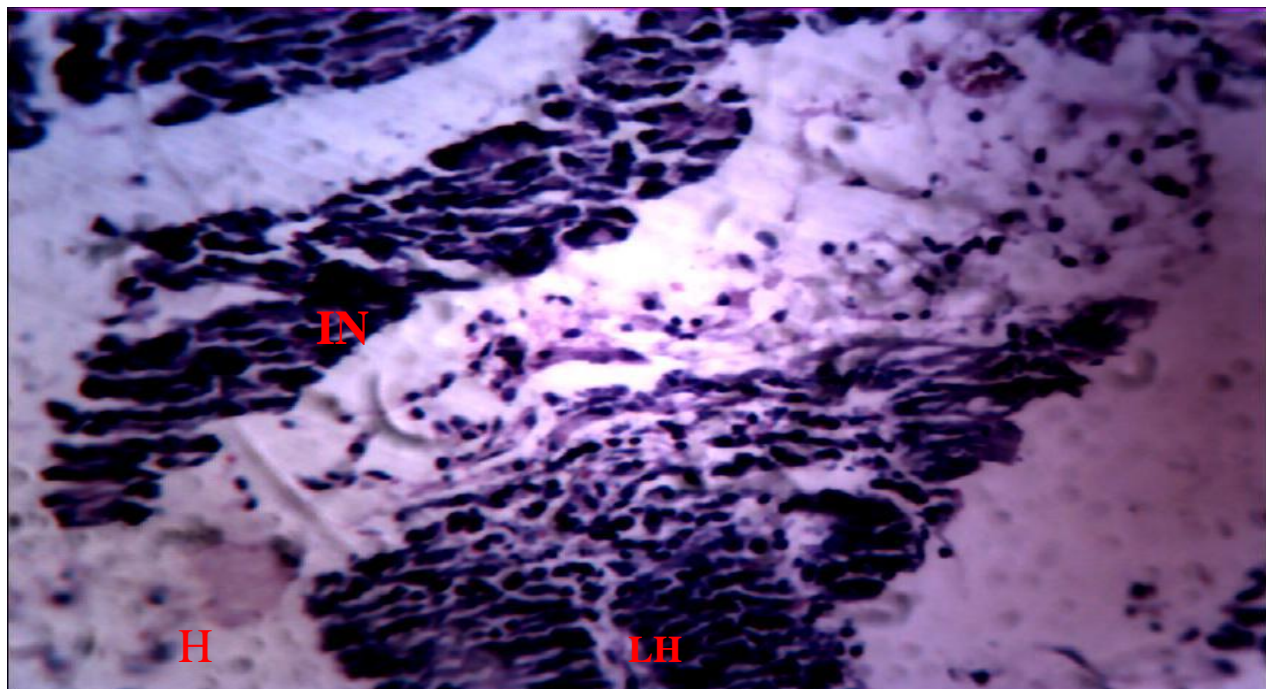


Plate II: Photomicrograph of pancreas from Alloxan-induced diabetic Wistar rats (Positive control) given 1mg/kg of glibenclamide orally for two weeks showing islet necrosis (IN), lymphocyte hyperplasia (LH) and haemorrhage (H) (H and E x 250).

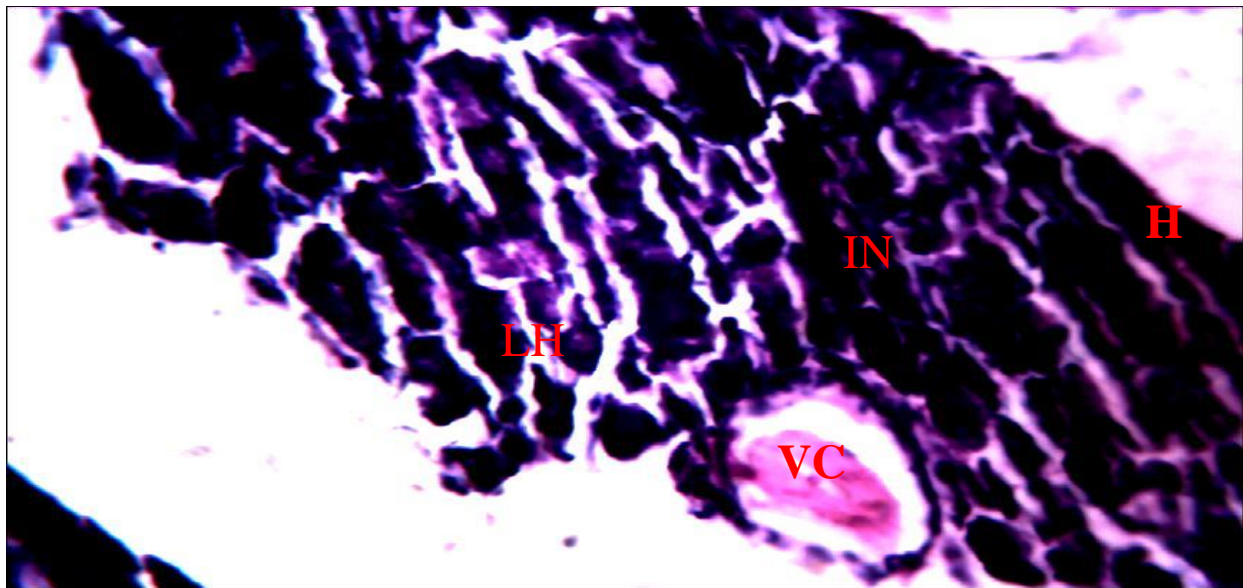


Plate III: Photomicrograph of pancreas from Alloxan-induced diabetic Wistar rats fed with 5% *P. sativum* seed supplement for two weeks showing islet necrosis (IN), lymphocyte hyperplasia (LH), vascular congestion (VC) and haemorrhage (H) (H and E x 250).

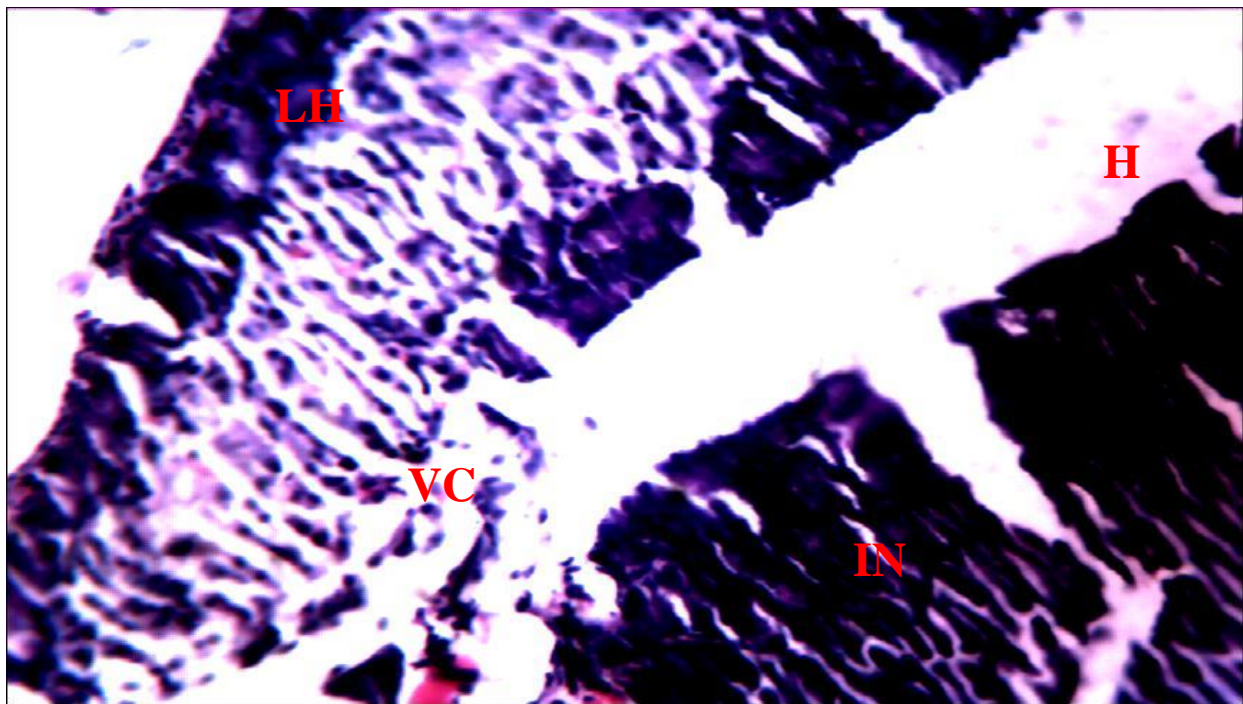


Plate IV: Photomicrograph of pancreas from Alloxan-induced diabetic Wistar rats fed with 10% *P. sativum* seed supplement for two weeks showing islet necrosis (IN), lymphocytes hyperplasia (LH), vascular congestion (VC) and haemorrhage (H) (H and E x 250).

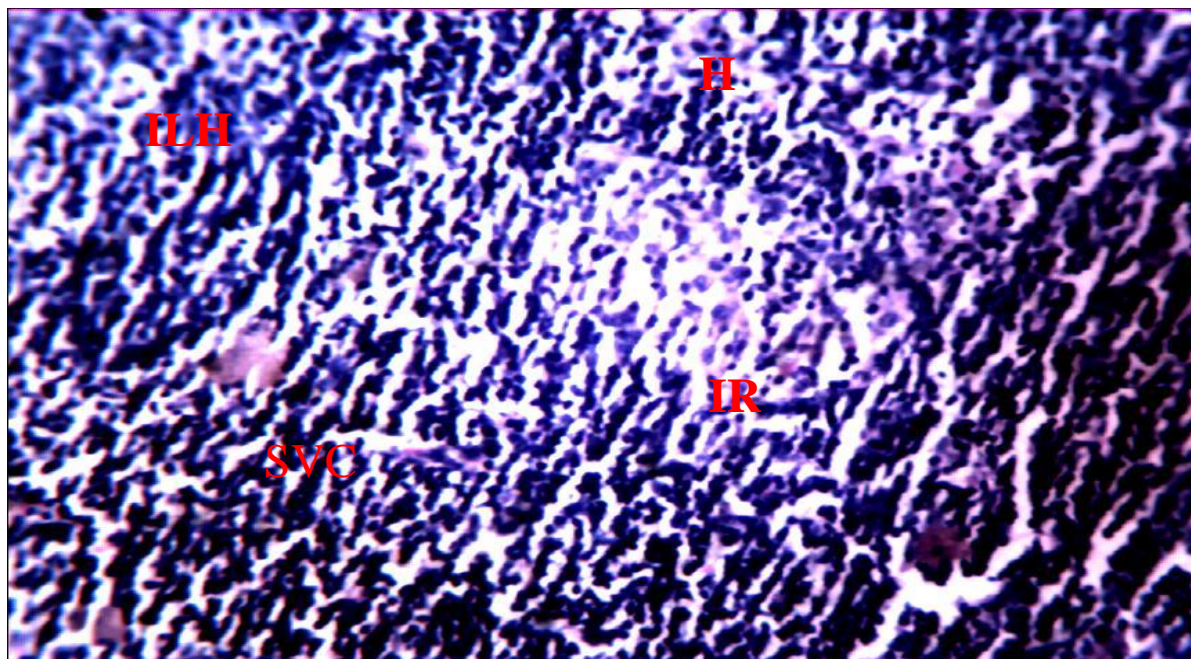


Plate V: Photomicrograph of pancreas from Alloxan-induced diabetic Wistar rats fed with 20% *P. sativum* seed supplement for two weeks showing haemorrhage (H), islet regeneration (IR), slight vascular congestion (SVC) and lymphocyte hyperplasia (H and E x 250).

DISCUSSION

This study was carried out to investigate the effect of *Pisum sativum* supplement in different percentages (5%, 10% and 20%) on blood glucose level, serum lipid profile, liver enzymes and pancreas of the experimental rats. The result obtained from the present study showed that *P. sativum* used as a supplement in the rat feed, reduced blood glucose level in the treated groups when compared with the diabetic untreated group with even more potent antihyperglycaemic effect on the treatment groups than in the positive control glibenclamide (1 mg/kg). This could be due to the constituents of the supplement as revealed by phytochemical screening especially flavonoids, alkaloids, vitamins and saponins as revealed by phytochemical screening, which might be responsible for the anti-diabetic effect [23]. This support the work of Tanko *et al.* [24] in which they used the ethanolic flower extract of *Newbouldia* leaves containing flavonoids and saponins, which was found to decrease blood glucose of the treated groups when compared to the control. *P. sativum* seeds are source of omega-3 fats in the form of alpha-linolenic acid and linoleic acid which are polyunsaturated fatty acids [25]. Polyunsaturated fatty acids are known to promote glucose uptake through GLUT4 transporters in adipocytes [25] thereby improving insulin sensitivity. This may be the possible mechanism through which the *P. sativum* supplement exert it hypoglycaemic effect. The hypoglycaemic effect seen in this study is in line

with the work of Oluwakemi *et al.* [27], in which they revealed the antidiabetic potentials of the aqueous and methanolic leaf extract of *Bougainvillea glabra* on Alloxan-induced diabetes mellitus and attributed it to the phytochemicals (alkaloids, flavonoids, saponin, and cardiac glycoside) in the extract, which are also present in *P. sativum* seed supplement (carbohydrates, cardiac glycosides, triterpene, alkaloids, tannins, saponins, flavonoids and steroids) respectively.

Lipid levels play an important role as a marker of oxidative stress. Diabetes mellitus has been linked to oxidative stress [28]. In this study, although there was a decrease in the mean total cholesterol, triglycerides, high density lipoprotein cholesterol and low-density lipoprotein cholesterol within the treated groups when compared with the diabetic untreated group, However, it was not statistically significant. The non-significant decrease seen may be due to the duration of administration. This result is in agreement with the findings of Seven *et al.* [29], in which they investigated the effect of Vitamin E supplementation on oxidative stress in streptozotocin-induced diabetes in rat liver and plasma, and found that Vitamin E supplementation for four weeks, significantly lowered lipid peroxidation in diabetic livers when compared to the control group. This study is also in agreement with the findings of Akomas *et al.* [30], where a significant decrease in the serum levels of triglyceride, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and in high density lipoprotein cholesterol in diabetic rats

treated with ethanol extract of *Ficus sur* leaves in Alloxan-induced diabetic rats, was reported.

Elevated concentration of ALT, AST and ALP in the serum is used in assessment of liver damage [31]. The result obtained from this study showed significant increase in the serum concentration of ALT and AST in the diabetic rats fed with 20% of *P. sativum* supplement when compared with the diabetic untreated group. The significant increase in the serum concentration of liver enzymes is in agreement with the work of Cryer *et al.* [32], in which they found out that excessive elevation of liver enzymes was due oxidative stress induced liver damage. The increase despite the supplement administered in the treated groups could be due to the potent hypoglycaemic effect of *P. sativum* supplement which could have exacerbated the liver damage from the diabetic induction due to cellular damage as observed by McAnuff *et al.* [33]. This study is in contrast with the work of Kim *et al.* [34]; they reported significant decrease in the serum concentration of ALT and AST in streptozotocin-induced diabetic rats fed with bitter yam (*Dioscorea polygonoides*) extract, which might be due to duration of administration and also constituent of the bitter yam.

Histopathological studies are usually done to assay the integrity of certain organ or tissue [22]. The photomicrographs sections of pancreas from diabetic untreated rat showed intense islet cell necrosis when compared with the *P. sativum* supplemented groups in which slight islet cell necrosis, intense lymphocyte hyperplasia; vascular congestion, haemorrhage and regeneration of islet cells were observed.

The lymphocytes hyperplasia seen in the *P. sativum* supplemented groups may explain the protective effect of lymphocyte against infection that can cause damage to tissues. Lymphocytes offered protection against infections [35]. The vascular changes observed may be due to healing process. This finding could suggest a hepato-protective effect of the supplement thereby ameliorating the islet cells necrosis, and a possible effect on islet cells regeneration. This study agrees with the work of Jain and Jain [36]. They found out that, Vitamin C and E supplementation prevent cellular damage from oxidative stress in diabetic rats. The regeneration of the islet cells may be attributed to vitamin C and tannins contents of *P. sativum* [23], which might have enhanced the recovery of the residual beta cells, possibly the gradual improvement seen in the photomicrographs of the pancreas from the treated groups when compared with the diabetic untreated, since tannins has found to possess anti-inflammatory and anti-microbial activities as reported [37].

CONCLUSION

P. sativum supplementation on Alloxan-induced hyperglycaemic Wistar rats reduced blood glucose level, increased concentrations of liver enzymes with no significant change in serum concentration of lipids.

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