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Original Research Article

Effect of Pre-mixed Extract of *Adansonia digitata* and Garlic on Streptozotocin (STZ) Induced Hyperglycemic Rat Model

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Abstract: The study investigated the effect of *Adansonia digitata* and garlic on Blood glucose level in streptozotocin induced diabetic rats. Twenty (20) albino rats were divided into four groups (GI, II, III, and IV) of five rats each and their fasting blood glucose was noted prior to inducement with diabetes using streptozotocin (STZ) at dose of 50mg/kg body weight. GI serves as diabetic control, receives no extract, while GII, GIII and GIV were respectively administered with 500mg/Kg *Adansonia digitata* extract, 400mg/Kg garlic extract and 500mg/Kg *Adansonia* – Garlic premix orally for 12 days. The serum blood glucose was monitored at interval of three days, after which the rats were allowed for nine days without extract with periodic record of their blood glucose at interval of three days. The fasting blood glucose in each group 24 hours after streptozotocin (STZ) injection was significantly higher ($P<0.05$) when compared Zero hours (before induction). The periodic Blood glucose levels were significantly lower ($P<0.001$) in the Group treated with 500mg/kg of *Adansonia digitata*, 400mg/kg of garlic and 500mg/kg of the premix extract compared to the corresponding period of GI. On withdrawal of the extracts, blood glucose of the diabetics rats increase. It may be concluded that both *Adansonia digitata* and garlic may possess hypoglycemic activity which could be due to their phytochemical and mineral contents.

Keywords: Diabetes mellitus, *Adansonia digitata*, garlic, hyperglycemic.

INTRODUCTION

Diabetes mellitus, is a complex disorder characterized by chronic hyperglycemia and glucose intolerance (Ahmad *et al.*, 2019), resulting from malfunction in insulin secretion and/or insulin action, it is the most common serious metabolic disease in the world, affecting hundreds of millions and having incidence rate of about 1 % in industrialized countries (Gwarzo *et al.*, 2014). There are two types of diabetes, (Rashid *et al.*, 2014),

1. Type I or Insulin dependent diabetes mellitus is an autoimmune genetic disease resulting from an absolute immune genetic disease resulting from an absolute deficiency of insulin due to destruction of pancreatic β-cells.
2. Type 2 or Non-insulin dependent diabetes mellitus is a multifactorial disease which is characterized by insulin resistance associated not only with hyperinsulinemia and hyperglycemia but also with atherosclerosis, Hypertension and abnormal lipid profile, collectively called syndrome X (Ibrahim *et al.*, 2021).

Medicinal Plants

Medicinal plants are those plants that are used (parts, extract etc) in treating and preventing specific ailments and diseases that affect human beings. Hence the important role of medicinal plants in health care delivery (services) cannot be over emphasized. There are so many types of medicinal plants with antidiabetic activity (Arumugam, Manjula and Paari, 2013).

Some Medicinal plants having anti-diabetic activity

S/n	Plant name	Family	Extract	Activity
1	*Alangiumlamarckii*	Alangiaceae	Alcoholic	Antidiabetic
2	*Albiziaodoratissima*	Mimosaceae	Methanol	Antidiabetic
3	*Cyclocaryapaliurus*	Cyclocaryaceae	chloroform, ethyl acetate & n-butanol	Hypoglycemic
4	*Embeliaribes*	Myrsinaceae	Hexane	Antidiabetic
5	*Marrubium vulgare*	Lamiaceae	Methanol	Hyperglycemia
6	*Psidiumguajava*	Myrtaceae	Ethanol	Antihyperglycemic
7	*Cassia auriculata*	Caesalpiniacae	Aqueous	Antihyperglycemic

Garlic (*Allium sativum*)

Garlic (*Allium sativum L., Liliaceous, Tafarnuwa*) is a common spicy flavouring agent used since ancient times (Ahmad *et al.,* 2019). It has been grown around the world, from Mediterranean climates to Siberia. Its medical and magical powers were described on the walls of ancient temples. It is considered a warm, bitter herb with particular effects on the Large intestine, Spleen and Stomach meridians. It is used to lower blood pressure, to treat parasitic infections, food poisoning and tumours, and as a mild anticoagulant (Chadare *et al.,* 2009). It is a remarkable plant which has multiple beneficial effects. The extract of garlic and its component, S-allylcysteine sulfoxide, significantly decreased blood glucose concentration. Commercially available garlic preparations in the form of garlic oil, garlic powder, and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile (Sajid *et al.,* 2014).

Adansonia digitata

Adansonia digitata (Baobab) is a deciduous tree and belongs to the plant family called *Bombacacee*. Baobab contains a number of substances usually employed for the treatment of numerous diseases in the African traditional medicine and for that reason it is also named “the small pharmacy”. The dry baobab fruit pulp possess particularly high values for carbohydrates, energy, calcium, potassium (very high), thiamine, nicotinic acid and vitamin C (very high). However, the vitamin C content of the bulk fruit pulp reportedly varies from 1623mg/kg in one tree to 4991mg/kg in another. Baobab fruit pulp has traditionally been used as an immune stimulant, anti-inflammatory, analgesic, antipyretic, febrifuge, and astringent in the treatment of diarrhoea and dysentery. The aqueous extract of baobab fruit pulp exhibited significant hepato-protective activity and as a consequence, consumption of the pulp may play an important part in human resistance to liver damage in areas where baobab is consumed (Sugandha, Parasharami and Rai, 2013). Taking cognizance that the pulp fruits of *Adansonia digitata* are traditionally consumed as a food sources as well as for medicinal purposes, such as management of diabetes mellitus in Hausa land.

This study evaluated the hypoglycemic activity of the *Adansonia digitata* and Garlic extract on blood glucose levels of STZ induced diabetic rats. As part of nutritional therapy, diabetic patients are advised to avoid high carbohydrate diets. Hence evaluation of hypoglycemic activity becomes imperative in order to establish any therapeutic benefit in the used of pulp in the management of diabetes mellitus in Hausa land.

This research was aimed to determine the hypoglycemic effect of *Adansonia digitata* and garlic pre-mixed extract. The observed reasons for these trends are poor clinic accessibility and drug availability, high costs of treatment and care, inadequate supplies of trained staff and equipment, use of alternative health care providers among others.

METHODOLOGY

Sample Collection

The fruit pulps of *Adansonia digitata* and *Allium sativum* (garlic) were purchased From Hotoro Danmarke market, Maiduguri road Kano State, Nigeria. The plant was identified and authenticated with herbarium number of 0297 by a specialist in the Department of Biological Sciences, Bayero University, Kano.

Experimental Animals

Twenty five albino rats weighing 100-180g were obtained from animal house, Biological science department Bayero University, Kano. The rats were maintained under good ventilation and standard conditions. The animals were feed with Niger feed (pelletized grower feed) manufactured by Ultra-Fine Food Company Nigeria LTD, Kano.

Extract preparation

The fruit pulps of *Adansonia digitata* were broken, and crushed without seeds with pestle and mortar, then sieved to obtain the powder. 100mg/ml of the *Adansonia digitata* extract was freshly prepared with water. Garlic (*Allium sativum*) cloves were separated, peeled and crushed. The crushed cloves were soaked in distilled water for 24hours. The extract was filtered and ready for study.

Streptozotocin Induction

A Stock solution of streptozotocin was prepared by dissolving streptozotocin powder (1g) in 20ml of saline water to give a stock concentration of approximately 50mg/ml. All the animals (albino rats) were fasted for about 14hours before the inducement of

streptozotocin (STZ) solution. The STZ injection was administered immediately after the preparation of the solution. All the doses of STZ were giving in 50mg/kg body weight of the experimental animals.

The blood glucose concentration was measured every three days from the day of induction to 21 days. The blood samples were collected from the tail and the blood glucose concentration determined by Accu-chek Active gluco-meter and its strip.

Principle of Accu-chek active gluco-meter
Glucose oxidase method:

When a drop of blood is apply on the strip, the glucose oxidase catalyses the conversion of glucose in the blood sample to hydrogen peroxide:

$$\text{Glucose} + O_2 \longrightarrow \text{Gluconic acid} + \text{Hydrogen peroxide } (H_2O_2)$$

In the presence of the enzyme peroxidase, the chromagen dye is oxidised by the hydrogen peroxide to produce a colour change on the fibre pad of the strip.

$$DH_2 \text{ (chromagen dye)} + H_2O_2 \longrightarrow 2H_2O + D \text{ (blue)}$$

Experimental protocol

Twenty five rats were distributed into five experimental groups. Each group consisted of five experimental rats in the beginning of the experiment.

- GI: Diabetic control rats induced with 50mg/kg of STZ without treatment.
- GII: Diabetic rats induced with 50mg/kg of STZ and treated with 500mg/kg of *Adansonia digitata* extract.
- GIII: Diabetic rats induced with 50mg/kg of STZ and treated with 400mg/kg of garlic extract.
- GIV: Diabetic rats induced with 50mg/kg of STZ and treated with 500mg/kg pre-mix of *Adansonia digitata* and garlic extract.

Histopathology

Procedure for histopathology

The biopsies of the pancreas of Wister albino rats were fixed with 10% formal saline, dehydrated with ascending grade of alcohol, cleared with toluene, infiltrated with molten paraffin wax. The microtome sections were stained with hematoxylin and eosin staining technique.

Statistics

Results were statistically analysed using one way analysis of variance (ANOVA). The P-value is <0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance follow Tukey-Kramer Multiple Comparisons Test. If the value of q is greater than 4.046 then P-value is less than 0.05.

RESULTS

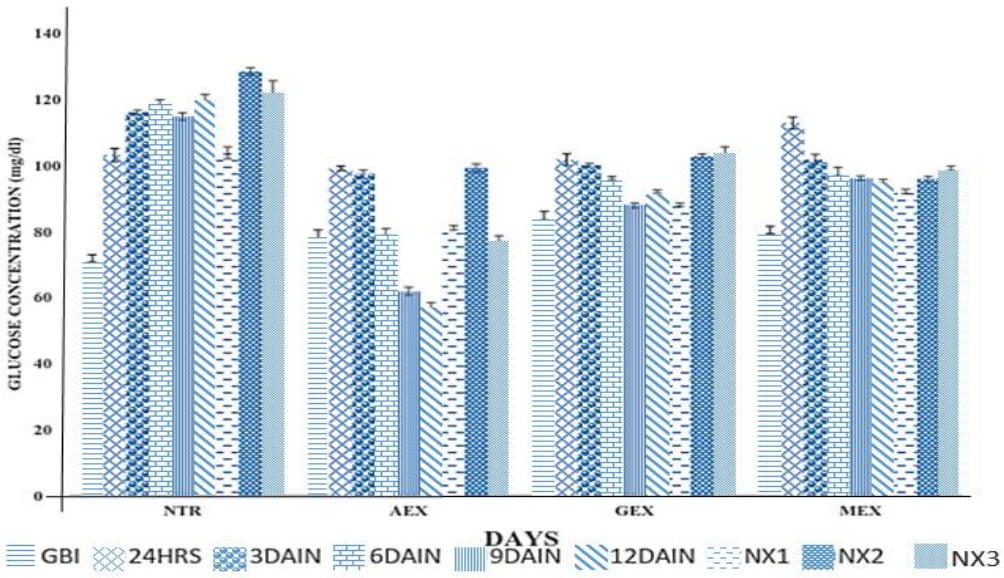

Figure 1: Periodic serum glucose concentrations of streptozotocin induced diabetic rats administered with *Adansonia digitata*, garlic and adanso-garlic premix for 21 days and three days after withdrawal of the treatment

Table 1: A significance difference of glucose level of a diabetic Rats of positive control (NTR), treated with pure *Adansonia digitata extract* (AEX), pure garlic extract (GEX), and premix of *Adansonia digitata* and garlic extract (MEX) before inducement

Comparison	Mean Difference	Q	P value
NTR VS AEX	-7.330	13.380	*** P<0.001
NTR VS GEX	-13.000	23.731	*** P<0.001
NTR VS MEX	-8.660	15.808	*** P<0.001
AEX VS GEX	-5.670	10.350	*** P<0.001
AEX VS MEX	-1.330	2.428	ns P>0.05
GEX VS MEX	4.340	7.922	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 2: A significance difference of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic 24hours after induction

Comparison	Mean Difference	Q	P value
NTR VS AEX	4.000	5.447	** P<0.01
NTR VS GEX	1.330	1.811	ns P>0.05
NTR VS MEX	-9.670	13.168	*** P<0.001
AEX VS GEX	-2.670	3.636	ns P>0.05
AEX VS MEX	-13.670	18.615	*** P<0.001
GEX VS MEX	-11.000	14.979	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 3: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic, 3 days after induction

Comparison	Mean Difference	Q	P value
NTR VS AEX	18.670	39.966	*** P<0.001
NTR VS GEX	16.000	34.250	*** P<0.001
NTR VS MEX	14.330	30.675	*** P<0.001
AEX VS GEX	-2.670	5.715	** P<0.01
AEX VS MEX	-4.340	9.290	*** P<0.001
GEX VS MEX	-1.670	3.575	ns P>0.05

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 4: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic, 6 days after induction

Comparison	Mean Difference	Q	P value
NTR VS AEX	39.000	82.374	*** P<0.001
NTR VS GEX	22.670	47.883	*** P<0.001
NTR VS MEX	20.670	43.658	*** P<0.001
AEX VS GEX	-16.330	34.491	*** P<0.001
AEX VS MEX	-18.330	38.716	*** P<0.001
GEX VS MEX	-2.000	4.224	* P<0.05

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 5: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic, 9 days after induction

Comparison	Mean Difference	Q	P value
NTR VS AEX	53.000	119.82	*** P<0.001

NTR VS GEX	26.670	60.294	*** P<0.001
NTR VS MEX	18.670	42.208	*** P<0.001
AEX VS GEX	-26.330	59.526	*** P<0.001
AEX VS MEX	-34.330	77.612	*** P<0.001
GEX VS MEX	-8.000	18.086	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 6: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic, 12 days after induction

Comparison	Mean Difference	Q	P value
NTR VS AEX	63.330	147.24	*** P<0.001
NTR VS GEX	28.330	65.867	*** P<0.001
NTR VS MEX	25.330	58.892	*** P<0.001
AEX VS GEX	-35.000	81.374	*** P<0.001
AEX VS MEX	-38.000	88.349	*** P<0.001
GEX VS MEX	-3.000	6.975	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 7: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic first day after removal of treatment

Comparison	Mean Difference	Q	P value
NTR VS AEX	22.700	48.864	*** P<0.001
NTR VS GEX	15.700	33.796	*** P<0.001
NTR VS MEX	11.700	25.185	*** P<0.001
AEX VS GEX	-7.000	15.068	*** P<0.001
AEX VS MEX	-11.000	23.679	*** P<0.001
GEX VS MEX	-4.000	8.610	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 8: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic, second day after removal of treatment

Comparison	Mean Difference	Q	P value
NTR VS AEX	29.330	63.686	*** P<0.001
NTR VS GEX	25.660	55.717	*** P<0.001
NTR VS MEX	32.660	70.916	*** P<0.001
AEX VS GEX	-3.670	7.969	*** P<0.001
AEX VS MEX	3.330	7.231	*** P<0.001
GEX VS MEX	7.000	15.199	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 9: A significance different of glucose level of a diabetic Rats of positive control, treated with pure *Adansonia digitata* extract, pure garlic extract, and premix of *Adansonia digitata* and garlic third days after withdrawal of treatment

Comparison	Mean Difference	Q	P value
NTR VS AEX	29.330	63.686	*** P<0.001
NTR VS GEX	25.660	55.717	*** P<0.001

NTR VS MEX	32.660	70.916	*** P<0.001
AEX VS GEX	-3.670	7.969	*** P<0.001
AEX VS MEX	3.330	7.231	*** P<0.001
GEX VS MEX	7.000	15.199	*** P<0.001

The P value is < 0.0001, considered extremely significant. Variation among column means is significantly greater than expected by chance.

Table 10: Insulin concentration after 21 days of experiment

GROUPS	INSULIN CONCENTRATION (ng/ml)
Normal control	0.270
GI (NTR)	0.260
GII (AEX)	0.384
GIII (GEX)	0.286

Histopathology

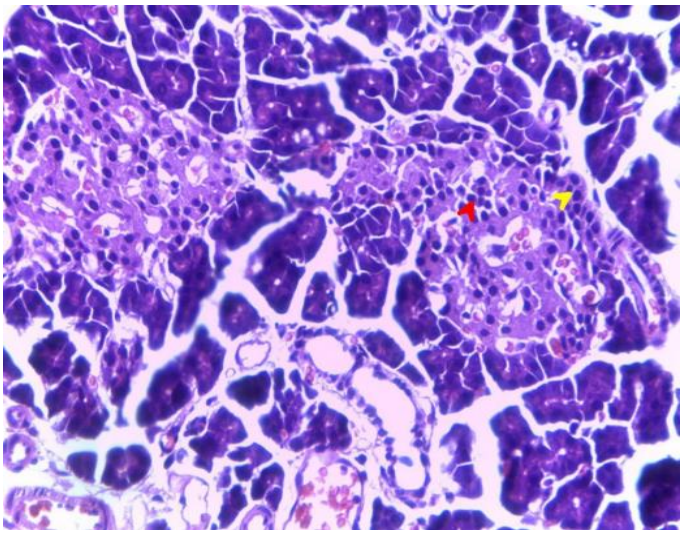

Figure 2a: Pancreas of a normal control rats shows alpha cells (yellow arrow) and 70% beta cells (red arrow) in the islet

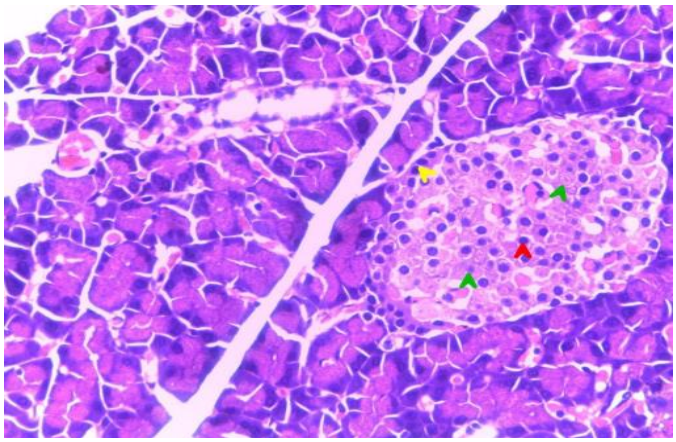

Figure 2b: Pancreas of diabetic rats with no treatment shows areas of beta cells depletion (green arrow) by the action of streptozotocin

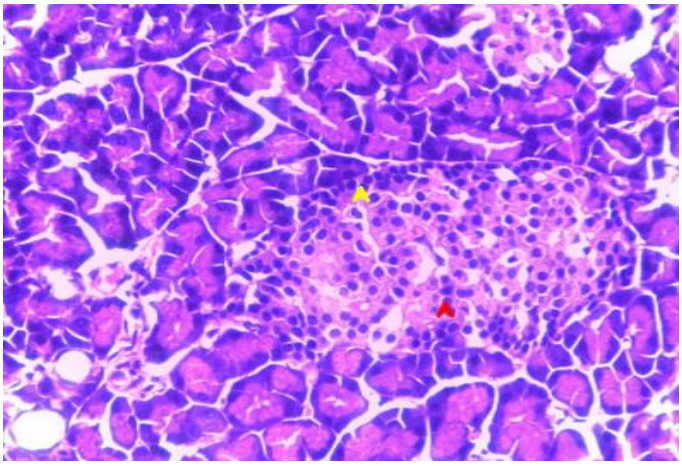

Figure 2c: Pancreas of diabetic rats treated with 500mg/kg of *Adansonia digitata* extract shows large number of alpha cells (yellow arrow) as compared to the beta cells (red arrow)

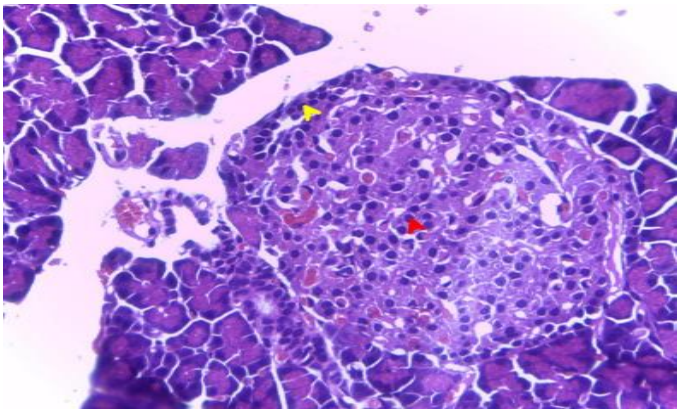

Figure 2d: Pancreas of diabetic rat treated with 400kg/kg of garlic extract shows large number of beta cells (red arrow) as compared to the alpha cells (yellow arrow)

Table 11: Fourier Transform Infrared Spectroscopy (FTIR) Result of Garlic

Peaks	Absorbance	Standard library	Class of compounds	Functional group
1	405.86 – 618.14	N-(2- Ethoxyphenyl)-N- (2-Ethylphenyl) Ethanediam nie	Ethers, Enolate, Emines, Alkylhalides	C-O C=C C – X
2	853.23 – 929.58	Poly(acrylamide, carboxyl modified, high carboxyl content	Carboxyl	C – OH COOH
3	1044.12 – 1122.59	Opium powder (morphine)	Amines,	C-N C-N-C C-N-C C
4	1254.96	Streptomycin	Oligosaccharide	
5	1416.33 - 1455.22	Erythromycin	Oligosaccharide	- OH
6	1505.43	Chondroitin sulphate	Glucosamine	- COO-, -OSO3-
7	1651.57	Regulaid	Alkenes	C == C
8	2149.94	Adenosine 5`- diphosphoglucose	Nucleotide	
9	2927.01	Poly(Dimethylsiloxane)	Alkane chains, Carboxylic	C- H, - OH
10	3401.08	Glycerol	Alcohol, Amide, Carboxylic, phenols	- OH

DISCUSSION

From the result of this research work, Figure 1 show a significant ($p<0.001$) increase in serum glucose concentration of NTR, AEX, GEX and MEX at 24HRS compared with zero hours (before STZ injection). That was due to the intramuscular induction of 50mg/kg body weight of streptozotocin, which destroy the beta cells of the pancreas that interfere with insulin

synthesis, as a result of that the concentration of glucose in the blood increases (Aliyu, 2015). Figure 2b of histopathology result show the decrease in the beta cells of the pancreas and this confirm the inducement of diabetes. A significant increase in serum glucose concentration across the figure in GI (Diabetic control without treatment) was due to the depletion of the β-cells by streptozotocin injected intramuscularly without treatment, which correspond to the work done by (Aliyu, 2015), Figure 2b of histopathology show the depletion of the beta cell, which conform the induction of diabetes in the experimental animals. Furthermore, a significant decrease in serum glucose level was observed across the figure with the exception of GI (NTR), (3DAIN, 6DAIN, 9DAIN and 12DAIN) compared to 24hrs glucose level after induction (24HRS), this could be due to the treatments, which lead to the regeneration of β- cells of the pancreas and enable the production of insulin as shown by Figure 2c and 2d of histopathology result. Moreover, in NX1, NX2 and NX3 (days after the withdrawal of treatment), the glucose level increase across the figure due to the withdrawal of treatment for 9days. All these clearly indicate the antidiabetics effect of garlic and *Adansonia digitata* extract, in contrary with observation done by Gwarzo *et al*., (2014) and Ashraf *et al*., (2011).

From the research work, Table 1-9 show a significant ($p<0.001$) differences in GI (NTR) compared with diabetic rats treated with 500mg/kg of *Adansonia digitata* extract (AEX), diabetic rats treated with 400mg/kg of garlic extract (GEX) and diabetic rats treated with 500mg/kg pre-mix of *Adansonia digitata* and garlic extract (MEX), due to treatment with pure *Adansonia digitata*, pure garlic and pre-mix of garlic and *Adansonia D*. extract respectively.

The insignificant differences could be due to time variation in the measurement of serum glucose concentration by the researcher. All these show a clear indication that *Adansonia digitata* and garlic extract have hypoglycemia effect on streptozotocin (STZ) induced rats, similarly, as concluded by (Alhassan *et al.*, 2016). Plates 2c and 2d show the regeneration of the β- cells of the pancreas and this confirm the antidiabetic effect of *A. digitata* and garlic extract. Also table 10 confirm the production of insulin by *Adansonia digitata* and garlic treated groups (AEX and GEX) compared with insulin concentration in GI (NTR). A significant differences ($P<0.001$) between the groups could be as a result of variation in hypoglycemic effect between the extracts. Table 11 of FTIR analysis show functional groups related compound in garlic extract such as amines, ethers, glucosamine and phenols which correspond to the functional groups present in some diabetic drugs (Metformin, Actos and Sulfonylureas).

Moreover, figure 2d of the histopathology result confirm the hypoglycemic effect of garlic with more islet beta cells regeneration than *Adansonia digitata* but compared to Figure 2c, diabetic rats treated with 400mg/kg of garlic extract (GEX) have lower anti diabetic activity compared to diabetic rats treated with 500mg/kg of *Adansonia digitata* extract (AEX), this could be due to secretion of somatostatin (growth inhibitory hormone) by the delta cells of pancreas, which inhibit the secretion of insulin by the β-cells. The secretion of somatostatin could be due to high dose of garlic extract administed to the group of diabetic rats treated with garlic extract.

CONCLUSION

This research work confirm the hypoglycemic activity of *Adansonia digitata* and garlic extract. It observed that the premix of the extract reduced the hyperglycemic effect of diabetic. It concluded the present of some functional groups in garlic extract associated with hypoglycemic effect of the extract.

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