Original Research Article

Hepatoprotective Effects of Ethanol Extract of *Allium sativum* (Garlic) on Diabetes Induced Male Wistar Albino Rats.

ABSTRACT

Allium sativum has been used traditionally for its various medicinal properties, including potential hepatoprotective effects and its role in managing diabetes. Some studies on animal models, including rats, have investigated the effects of *Allium sativum* extract on liver health and diabetes.This study was designed to investigate the hepatoprotective effects of ethanol extract of *Allium sativum*(garlic) on diabetes induced male wistar albino rats. Sixty (60) healthy male wistar albino rats weighing 90g-120g were used. Diabetes was induced in the rats by a single intraperitoneal dose of 130 mg/kg of Alloxan monohydrate. The rats were grouped into six groups: Group A (Control), Group B (Untreated Diabetes-induced), Group C (Diabetes-induced treated with 5mg/kg glibenclamide) and Group D, E, F which are Diabetes-induced treated with 200mg, 400mg and 600mg of ethanol extract of *Allium sativum*respectively. After the treatment period, blood samples were collected through cardiac puncture from each of the rats for Liver Function Test using standard analytical procedures. The Liver Function Test revealed that administration of alloxan which induced diabetes in the rats, increased both liver enzymes levels (Alkaline Phosphatase, (ALP), Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) and bilirubin levels. It was observed that administration of garlic extracts to diabetic rats significantly reduced the levels of ALP, ALT, AST, D BIL and T BIL. The result of this study showed that the ethanol extract of*Allium sativum* (garlic) has remarkable

hepatoprotective activity in diabetes-induced male Wistar albino rats. The observed improvements in liver function indicate the potential therapeutic benefits of garlic in managing liver complications associated with diabetes and may be recommended to individuals with high blood sugar (hyperglycemia) as a good alternative medicine to a standard antidiabetic drug for treating diabetes.

Key words: *Allium sativum*, diabetes, hepato-protective, health.

INTRODUCTION

Medicinal plants have been widely used for the treatment of many ailments due to their minimal or no side effects when compared with synthetic drugs[1]. Several studies have reported their beneficial properties in the management of various ailments, especially diabetes [2, 3]. Considerable evidences showed that indigenous medicinal plants in Nigeria form an important component of the natural wealth of the country[4]. Most of these plants have been used indiscriminately by many local populations for managing various diseased states without actually knowing how reliefs brought about or its safety/toxicity risk [4]. The regulations guiding the proper use of these medicinal plants, such as identification of the correct species and verification of the presence purity and concentration of the required chemical compounds, are widely recognized [4].

Diabetes mellitus (DM) is a chronic disorder affecting carbohydrate metabolism and involving hyperglycemia, as a result of deficiencies in insulin release, action, or even both[2]. These range from defects that lead to resistance to insulin action to autoimmune destruction of the pancreatic

B-cells with subsequent insulin shortage [5]; and have since been categorized into Type I and Type II.DM, when not properly managed, can lead to a series of complications and consequently to injuries in various tissues and poor quality of life [6]. Diabetes mellitus remains one of the age-long chronic diseases of the human race and its frontiers are expanding by the day.

The liver, with its multiple functions, is one of the most important organs. It plays an active role in metabolism as it secretes bile that breaks down fats in the small intestine during digestion, stores and releases glucose and synthesizes different types of proteins. In addition, the liver converts harmful ammonia into urea, processes haemoglobin, clears bilirubin, fights infections and detoxifies medicines and other toxic chemicals. Liver diseases are one of the chief reasons of morbidity and mortality worldwide. Several factors, directly or indirectly, cause liver diseases like virus, exposure to drugs or chemicals, obesity or diabetes. Besides this, different autoimmune disorders and untreated liver diseases can lead to malignancy and liver cancer and eventually death [7].

Allium sativum is a perennial herb that belongs to the amaryllidaceae plant family. It is one of the most multipurpose medicinal plants used as a traditional herbal medicine to prevent and treat a broad range of diseases, including cardiovascular diseases, atherosclerosis, hyperlipidaemia, thrombosis, hypertension and diabetes [8]. However, its potential in protecting the liver has not been well explored. Hence, this study was aimed at exploring the helpatoprotective activities of ethanol extracts of *Allium sativum* in male wistar albino rats induced with diabetes.

MATERIALS AND METHODS

Sample Collection and Preparation

The *Allium sativum* (garlic) used for the study was purchased from Eke market in Awka, Anambra State, Nigeria. The garlic was peeled, chopped and dried under room temperature, milled into powder and was stored in an air-tight container, until when needed for extraction.

Preparation of Sample Extract

The pulverized sample (Exactly 500g) was soaked in 2 litres of 70% ethanol (by maceration) for 48 hours with constant stirring at an interval of two (2) hours. At the lapse of the 48 hrs, the solution was filtered using muslin cloth and then with whatmann No 1 filter paper and the filtrate gotten was concentrated using water bath at 50^{o}C to obtain the crude extract which was thereafter stored in an airtight container and kept in a refrigerator until it was needed.

Procurement of Experimental Animals

Sixty (60) mature male wistar albino rats weighing between (90-120g) were obtained from Chris Farm Ltd Mgbakwu, Awka, Anambra State. They were sorted, housed in standard cages with housing conditions of 12:12 light: dark cycles. They were fed with standard grower's mash pellets and water *ad libitum.* All the experimental procedures and protocols used for this study were in accordance with the guidelines and principles of Animal Research Ethics Committee of Nnamdi Azikiwe University, Awka.

Induction of Diabetes to the Experimental Animals

Diabetes was induced in the experimental animals by a single intra-peritoneal injection of 130mg/kg Alloxan monohydrate using distilled water as the vehicle.

Dose Preparation and Treatment

The hydro-ethanolic extract of *Allium sativum* was prepared with distilled water in three divided dose (200, 400 and 600) mg/kg, glibenclamide (5mg/kg) was used as a reference drug and distilled water was administered to the untreated group. The animals were grouped into six different groups of ten (10) rats per group per cage and administered the extract and drug for twenty eight (28) consecutive days with water per os and feed *ad libitum* as shown in table 1.

Table 1: Grouping and Dose Administration of Experimental Animals

Group	Treatment
A (Normal)	Un-induced plus distilled water
B (Diabetic untreated)	induced plus distilled water
C (Standard control)	induced plus standard drug (5mg/kg glibenclamide)
D (Treatment)	induced plus 200 mg/kg*Allium sativum*extract
E (Treatment)	induced plus 400 mg/kg *Allium sativum*extract
F (Treatment)	induced plus 600 mg/kg *Allium sativum*extract

Collection of Blood Sample for Bioassay

At the end of 28 days, the rats were fasted overnight and then anaesthetized with chloroform vapor, and sacrificed. A 5 ml sterile syringe with needle was used for collection of blood via cardiac puncture from each of the rats into well-labelled plain tubes and allowed to clot for 2 hours. The samples were centrifuged at 400rpm for 30 minutes to recover the sera which was used for biochemical assays.

Biochemical Assay

Total Bilirubin (T BIL), Direct Bilirubin (D BIL) as well as the activities of Alkaline Phosphatase (ALP), Aspartate Aminotranferase (AST) and Alanine Aminotransferase (ALT)

were carried out using standard enzyme kits sourced from Randox Laboratories Ltd., BT29 4QY, United Kingdom with strict adherence to manufacturer's instructions.

DATA ANALYSIS

The results obtained in this research were expressed as Mean ± S.D of triplicate determinations. One way analysis of variance (ANOVA) was carried out on the results and significance was accepted at $p<0.05$.

RESULTS

The result on the effect of *Allium sativum*extract on total and direct bilirubin is presented in figure 1. Result showed that induction of diabetes to the experimental animals significantly increases the total and direct bilirubin in the experimental animals. However, treatment with *A. sativum*extract at different doses considerably lowered the bilirubin levels; bringing it to almost normal.

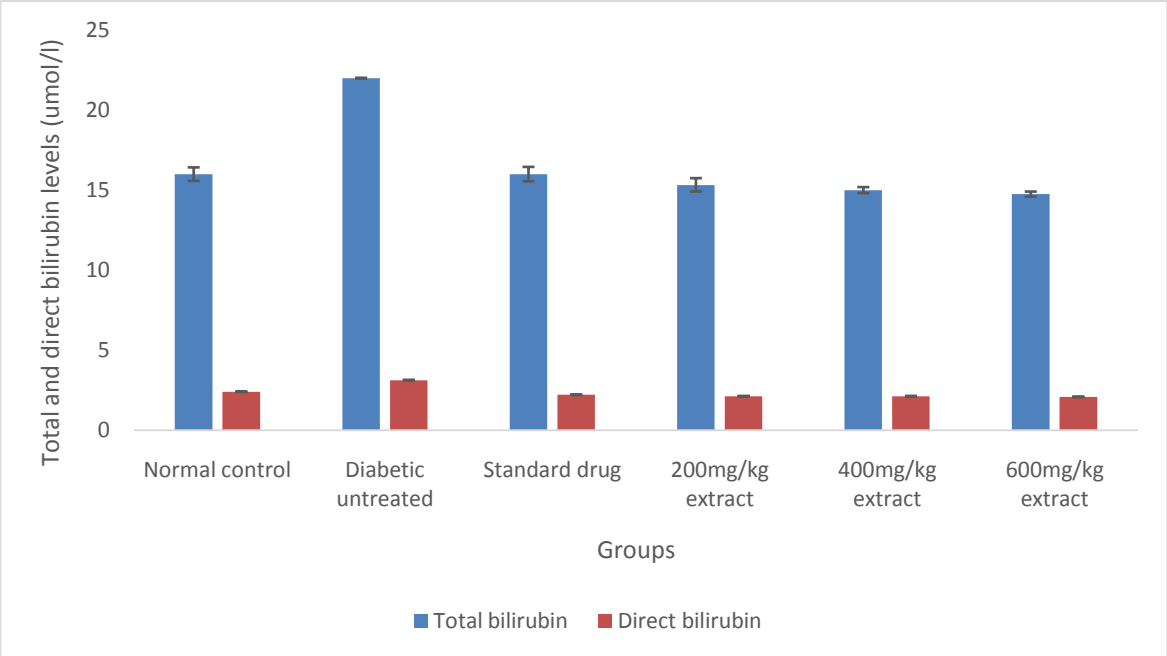

Figure 1: Effect of *Allium sativum*extract on the Total Bilirubin (T-BIL) and Direct Bilirubin (D-BIL) levels in alloxan-induced diabetic rats.

The result on the effect of *Allium sativum*extract on **Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) activities** is presented in figure 2. Result showed a marked decrease in the activities of serum ALT and AST in the extract treated groups compared with the control groups. However, a marked increase in the serum activities of ALP was observed in group treated with 600 mg/kg extract of *Allium sativum*compared with the untreated control group.

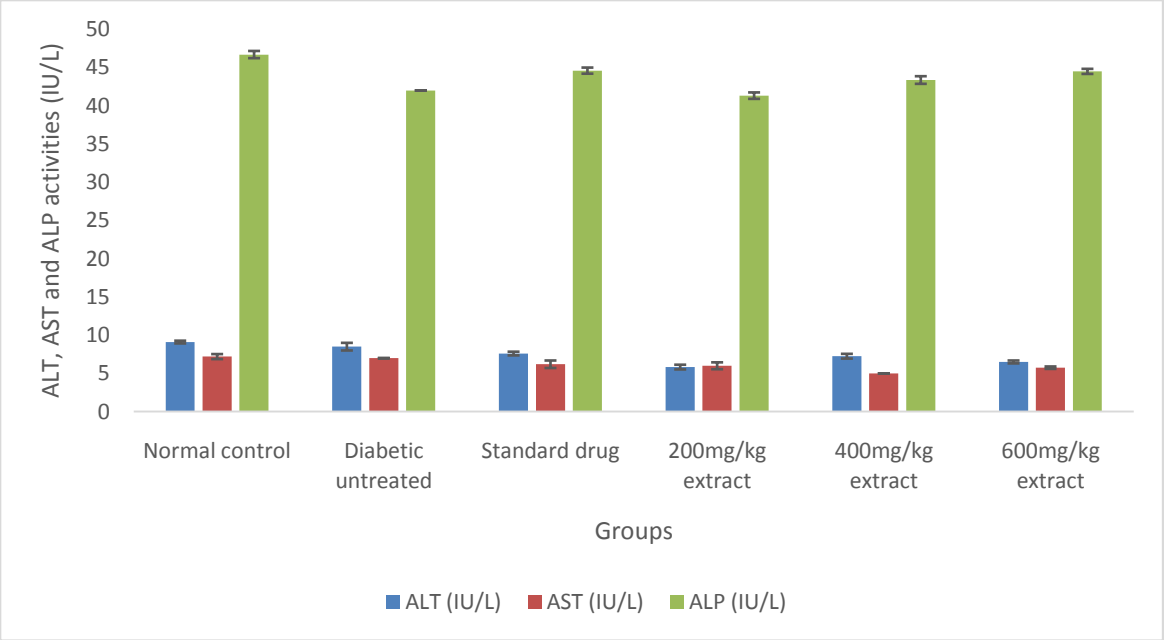

Figure 2: Effect of *Allium sativum*extract on Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) activities in alloxan-induced diabetic rats.

DISCUSSION

Since ancient times, medicinal plants have been used worldwide as traditional medicines in the management of various diseases. There are many studies exhibiting that some promising phytochemicals can be developed as an alternative for synthetic medicines for health problems [9]. Among these, many plants have been reported to have considerable hepato-protective activity. Still the search for new innovative drugs for the management of liver diseases is needed. Garlic is cultivated worldwide and is possibly effective for the management of various illnesses.

This study investigated the hepatoprotective effect *Allium sativum*extract in alloxan-induced diabetic rats to verify its health promoting abilities.

The liver is the second largest and one of the most important organs in our body which plays a significant role in regulating different biological processes. The liver has diverse functions such as metabolism, synthesis of many important biomolecules, detoxification, secretion and storage, etc. Further, the maintenance, performance, and regulation of body homeostasis is helped by the liver.

Almostallthebiochemicalpathwaysincludinggrowth,fightingagainstdisease,nutrientsupply,energy provision and reproduction, metabolism of carbohydrate, protein and fat, detoxification, secretion of bileandstorageofvitaminsarecarriedoutbyliver,whichalsohelpsinthedetoxificationandremoval of foreign substances. Hence, the liver is the prime target for attack by foreign substances which leads to liver disorders and damage. These days, liver diseases are a focus of more research because they cause notable morbidity and mortality worldwide. Some specific reasons of liver diseases include exposure to chemicals, excess consumption of alcohol, infections and autoimmune disorders [10]. It is well-known that induction of alloxan can lead to excessive formation of ROS which results in oxidative damage, lipid peroxidation and consequently diabetes.

Bilirubin is a tetrapyrrole and a breakdown product of heme catabolism. It is an important metabolite of heme (ferroprotoporphyrin IX), a coordination complex that serves to coordinate iron in various proteins. Its overproduction or defects in uptake and conjugation have been linked to jaundice, among several other ailments. As depicted in figure 1, induction of alloxan in the experimental animals caused a significant increase in the levels of total and direct bilirubin in the experimental animals. However, treatment with extract of *Allium sativum* was able to mitigate

this effect; bringing its levels to almost normal (15µmol/L). This findings is consistent with the reports of Aproku*et al.*[11]and Akhtar *et al*. [12].

Elevated or abnormal levels of liver function enzymes are considered to be significant markers of liver damage. In the current study, induction of alloxan was found to increase the levels of ALP, ALT and AST significantly, as compared to control group. However, our results also indicated that treatment of alloxan-induced animals with garlic extract caused a significant decrease of ALT, AST and ALP enzyme activities. Therefore, our results indicate that garlic extract has a significant hepato-protective potential. The findings of current study were in accordance with a previous study which has also confirmed that levels of AST and ALT became increased considerably after CCl4 treatment, and this was suppressed by aged black garlic treatment [13]. Naji *et al*. [14] made similar assertion that the administration of a single garlic clove resulted in a significant decrease of serum ALP, AST and ALT in stressed rats. Thus, this study affirms the claims and proposes that administration of garlic extract can have some significant role in prevention of liver damages occasioned with the administration of alloxan or any other chemicals.

Conclusion

Garlic is commonly used as a food additive and traditional medicine since ancient times. Our findings indicated that garlic extract possesses substantial hepato-protective activity which could significantly ameliorate liver damage, especially in diabetic patients.

REFERENCES

1. Onwubuya, E. I. and Oladejo, A. A. (2022). Possible Effects of Free Radical Scavengers on Dissolution of Inflammatory Granuloma: An Experience with Wistar Rats. Current Research in Complementary & Alternative Medicine, 5: 149.
2. Ezeigwe O. C., Oladejo, A. A. and Iloanya E. L. (2022). The Impact of *Citrus aurantium* Fruit Juice on Bodyweight and Haematological Parameters of Wistar Rats. *International Journal of Research and Reports in Hematology*,5(2): 56-67
3. Onwubuya, E. I., Ogbunugafor, H.A., Okafor, C.S. and Oladejo, A. A. (2021). Acute and Chronic Anti-inflammatory effect of *Bryophyllumpinnatum*Leaf Extract on Wistar Rats. *European Journal of Medicinal Plants*, 32(6): 6-14
4. Yakubu, M.T., Akanji, M.A and Oladiji, A.T. (2007). Haematological Evaluation in Male Albino Rats following Chronic Administration of Aqueous Extract of FadogiaAgrestis stem. *Pharmacognosy Magazine.* **3**(1): 34-38.
5. Saleh, A. A., Shehwaz, A., Ahmad, A., Amjad, A. K., Faris, A., Mohammed, A. A. and Arshad, H. R. (2020). Hepatoprotective Effects of Garlic Extract against Carbon Tetrachloride (CCl4)-Induced Liver Injury via Modulation of Antioxidant, Anti-Inflammatory Activities and Hepatocyte Architecture. Appl. Sci. 10, 6200.
6. Kuang, M.T., Li, J.Y., Yang, X.B., Yang, L., Xu, J.Y., Yan, S., Zhou J. (2020). Structural Characterization and hypoglycemic effect via stimulating glucagon-like peptide-1 secretion of two polysaccharides from dendrobiumofficinale: Available at www.sciencedirect.com>article>pii.
7. Sivakrishnan, S. Liver diseases—An overview. World J. Pharm. Pharm. Sci. 2019, 8, 1385–1395.

8. Anwar,S.;Younus,H.InhibitoryeffectofalliinfromAlliumsativumontheglycationofsuperoxidedismutase. Int. J. Biol. Macromol. 2017, 103, 182–193.
9. Oladejo, A.A., Onwubuya E.I., Ogbunugafor H. A., Okafor, C.S. and Ogbodo C. U. (2021). Inhibitory Effects of *Bryophyllumpinnatum*Leaf Extract on Inflammatory Biomarkers. *Journal of Advances in Medical and Pharmaceutical Sciences,* 23(2): 7-13.
10. Adevusi, E.A.; Afolayan, A.J. A review of natural products with hepatoprotective activity. J. Med. Plant Res. 2010, 4, 1318–1334.
11. Aprioku, J. S. and Amah-Tariah, F. S. (2017). Garlic (Allium sativum L.) Protects Hepatic and Renal Toxicity of Alloxan in Rats. Journal of Pharmaceutical Research International , 17(6): 1-7
12. Akhtar, M.T., Safdar, T.A.S., Saadia, M., Batool, R.Q.S., Mustaqeem, M., Shaukat, U.A., Kanwal, F. and Cai, R. (2023). Antioxidant, hypoglycemic,antilipidemic and protective effect of polyherbal emuldion on Alloxan-induced Diabetic Rats.American Chemical Society, 8(24): 21642-21652.
13. Shin, J.H., Lee, C.W., Oh, S.J., Yun, J., Kang, M.R., Han, S.B., Park, H., Jung, J.C., Chung, Y.H and Kang, J.S. (2014). Hepatoprotective Effect of Aged Black Garlic Extract in Rodents. *Toxicological Research.* **30**: 49-54.
14. Naji, K.M., Al-Shaibani, E.S., Alhardi, F.A., Al-Soudi, S.A. and D Souza, M.R. (2017). Hepatoprotective and antioxidant effects of single clove garlic against CCL_4-induced hepatic damage in rabbits. *BMC Complementary and Alternative Medicine.* **17**:1-12.