# Modulatory Role of Cabbage (*Brassica Olaeracea*) Supplement on Blood Glucose and Some Physiological Profiles on Alloxan Induced Diabetic Wistar Rats

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## Abstract
This study was designed to investigate the role of cabbage supplement on blood glucose and some physiological profiles on alloxan induced diabetic Wistar rats. A total of twenty five Wistar rats of both sexes weighing 100 – 150 g were used for the study. The animals were randomly allocated into five groups of five rats (n = 5 rats/group). Group one consisted of diabetic rats given distilled water 1ml/kg and served as the negative control. Group two was diabetic rats that received 5mg/kg b/w of glibenclamide orally and served as positive control. While, groups three, four and five were diabetic rats that received 10, 25 and 50% cabbage supplement, respectively. The experiment lasted for thirty days. Blood glucose and some physiological parameters including lipid profile, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP), were measured in all rats. Blood glucose level was significantly ($p < 0.05$) reduced in treated diabetic rats with cabbage supplement four weeks (108.80 ± 9.75 mg/dl for 10%, 95.20 ± 16.65 mg/dl for 25% and 59.80 ± 5.88 mg/dl for 50% cabbage) as compared to the diabetic control (281.80 ± 3.65 mg/dl) rats. In addition, serum total cholesterol, triglyceride, and low-density lipoprotein (LDL) were significantly decreased ($p < 0.05$) while high-density lipoprotein (HDL) was increased ($p < 0.05$) in treated than in diabetic control groups. Furthermore, feed supplementation with cabbage caused a significant increase ($p < 0.05$) in serum levels of AST and ALT but decrease ($p < 0.05$) in ALP enzymes. The results of this study suggest that cabbage when given as a supplement has hypoglycaemic and antihyperlipidaemic properties and thus its beneficial effect in the management of diabetes mellitus may be considered.

**Keywords:** Cabbage, Blood glucose level, Lipid profile, Liver enzymes

## Introduction
Diabetes is a devastating non-communicable disease that occurs due to the failure of the pancreas to produce enough insulin or when the body cannot use the insulin it produces effectively[1]. Diabetes Mellitus (DM) is a global health care menace that may reach pandemic levels by 2030[2]. About 80% of the total adult diabetics are in developing countries and the greatest concern is the growing incidence of Type 2 Diabetes at a younger age including some obese children even before puberty affecting the productive years of their lives[3]. Considerable evidence has seen diabetes changing into an epidemic in many developing countries with an estimated prevalence of 1% in rural areas of Africa and prevalence in Nigeria ranging from 0.65% in rural Mangu in the North to 11% in urban Lagos in the Southern part of the country[4][5]. Diabetes mellitus is a disease that is waging war against the well being of humans and may probably be due to drastic lifestyle changes accompanying urbanisation and westernisation in developing countries[6]. Individuals with diabetes are more likely to be hospitalized with cardiovascular disease, end stage renal disease and most frequently, non traumatic lower limb amputation compared to the general population[7].

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The number of individuals with diabetes has been increasing due to population growth, ageing, urbanization, and increasing prevalence of obesity and physical inactivity[8]. An estimated 415 million adults are said to be diabetic globally and the figure is expected to rise up to 642 million by 2040 [9]. The most important distinctive feature of diabetes is an elevated blood glucose concentration, but this abnormality is just one of a number of biochemical and physiological changes that occur[10]. Hypercholesterolemia and hyper triglyceridemia are common complications of diabetes mellitus[11]. The treatment of diabetes mainly involves the use of hypoglycaemic drugs in addition to insulin but the unwanted side effects of these drugs prompted a demand for new compounds for the treatment of diabetes[12]. The drive for change from orthodox to herbal medicines is to an extent due to the adverse reactions, undesirable side effects of synthetic drugs, the cost of buying modern antidiabetic drugs, which is beyond the reach of the lower class citizens and the belief that natural products are safer to the biological systems [13].

Cabbage (*Brassica oleracea*) is locally called *Kabeji* in Hausa language and *Akojopo* or *Jaleji* in Yoruba language. It is an important vegetable crop of the *Brassicaceae* family consumed all over the world. It is popular probably due to its low price and availability at local markets richness in phytochemicals such as polyphenolics, glucosinolates, carotenoids, and vitamin C. It consists of a wide range of important vegetable and fodder crops which are excellent sources of fibers that help prevents constipation, reduces the risk of colorectal cancer and helps to reduce blood sugar and blood cholesterol levels, thereby reducing the risk of heart disease and diabetes [14] [15]. Ethanolic extract of cabbage, has demonstrated significant hepatoprotective activity which justifies its use as a hepatoprotective agent as a result of the presence of biologically active phytoconstituents [16].

## Materials and Methods

### *Chemicals*

All chemicals and drugs used (Alloxan monohydrate, chloroform, ethanol, formalin, Glibenclamide and alloxan were purchased from (Sigma Chemical Company St. Louis U.S.A.).

### *Plant Material*

Fresh cabbages were purchased in April 2014 from Kubani farm, Zaria, Kaduna State. Authentication and identification was done by the Taxonomist in the Herbarium unit of Department of Biological Sciences, Ahmadu Bello University, Zaria and a voucher number (43382) was given for future reference.

### *Animals*

A total of twenty five albino Wistar rats of both sexes weighing 100-150 g were used for this study. The animals were obtained from the Animal House of Department of Human Physiology, Ahmadu Bello University, Zaria. They were randomised into experimental and control groups and were kept in polypropylene cages. Standard animal feed made of pellets from grower's mash were provided to the animals. The rats were allowed access to drinking water *ad libitum* throughout the period of the study.

### Preparation of Cabbage Supplement

Fresh leaves of cabbage was shed dried, ground, weighed and added to the rat feed (grower's mash) in 10, 25 and 50% percentage proportions.

### Experimental Induction of Diabetes Mellitus

Diabetes was induced by single intraperitoneal injection of alloxan monohydrate at a dose of 150 mg/kg body weight dissolved in 0.9% cold normal saline solution. The rats were fasted for 16 - 18 h before induction [17]. The rats were treated with 20% glucose solution orally for 6 hrs after induction and were then kept for the next 24 hrs on 5% glucose solution bottles in their cages to prevent hypoglycaemia [18]. After 72 hrs of alloxan treatment, venous blood was collected from the tail of the rats and those having fasting blood glucose level of ≥ 200 mg/dL were considered to be diabetic.

### Experimental Design

The study was carried out on alloxan induced Wistar rats. The animals were fasted for 16-18 hrs with free access to water prior to the induction of diabetes. After induction, the animals were randomly divided into 5 groups of 5 (n=5) animals as follows:

**Group 1: (Negative control):** Diabetic rats which were given 1ml/kg distilled water for four weeks.

**Group 2: (Positive control):** Diabetic rats that received 5mg/kg b/w of glibenclamide orally daily for 4 weeks.

**Group 3:** Diabetic rats that received 10% cabbage supplement for 4 weeks.

**Group 4:** Diabetic rats that received 25% cabbage supplement for 4 weeks.

**Group 5:** Diabetic rats that received 50% cabbage supplement for 4 weeks.

**Determination of Blood Glucose Levels and Physiological Profiles**

All venous blood samples were collected from the tail of the rats at weekly intervals for 4 weeks respectively. Fasting blood glucose levels was determined using glucose oxidase method [19] using a digital glucometer (Accu-Chek Advantage, Roche Diagnostic, Germany). The results were expressed in mg/dL [20]. Four weeks after the experimental period, all animals were sacrificed and blood samples drawn by cardiac puncture. The blood samples were collected in Eppendorf tubes, allowed to clot and the serum separated by centrifugation using Denley BS400 centrifuge (England) at 3000 g for 10 minutes. The supernatant (serum) collected was then used for lipid profile determination (serum total cholesterol, triglyceride, high density lipoprotein and low density lipoprotein) using the methods of [21], [22], [23] and [24] respectively and also for liver enzymes analysis (serum aspartate aminotransferase and alanine aminotransferase using the methods of [25] and alkaline phosphatase using the method described by [26].

**Statistical Analysis**

All data were expressed as mean ± SEM. The data obtained were statistically analysed using analysis of variance (ANOVA) with *Turkey's* multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis were evaluated using SPSS version 17.0 software. The values of $P < 0.05$ were considered significant.

## Results

Figure 1 showed the result of cabbage supplementation (10%, 25% and 50%) on serum total cholesterol, triglyceride, high-density lipoprotein and low-density lipoprotein in alloxan induced diabetic Wistar rats. The result showed significant ($P < 0.05$) increase in blood glucose level in weeks 1, 2, 3 and 4 in the positive control group that received only alloxan compared with the negative control group that received only normal saline (week 1: 410 ± 46.67 vs. 210 ± 22.98; week 2: 379.2 ± 6.67 vs. 262 ± 13.50; week 3: 347.4 ± 11.96 vs. 162.2 ± 13.77; week 4: 281.8 ± 3.65 vs.93.2 ± 5.23). Blood glucose level significant ($P < 0.05$) decreased in the 10%, 25% and 50% cabbage treated groups at fourth week of cabbage intake with values of 108.80 ± 9.75 mg/dl for 10%, 95.20 ± 16.65 mg/dl for 25% and 59.80 ± 5.88 mg/dl for 50% cabbage supplement compared to the diabetic positive control group.

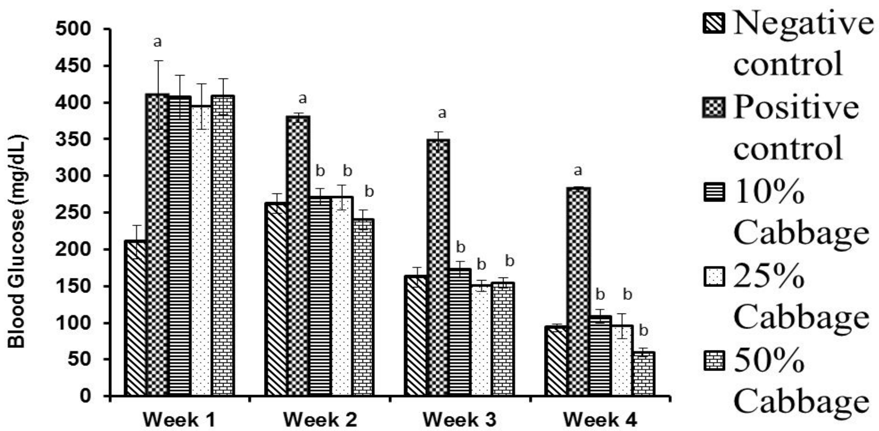


**Figure 1: The effect of cabbage supplement on blood glucose levels in alloxan induced diabetic rats. $^{a}P< 0.05$ vs. Negative control, $^{b}P< 0.05$ vs. Positive control.**

Figure 2 showed the effect of 10%, 25% and 50% cabbage supplementation on serum total cholesterol, triglyceride, high-density lipoprotein (HDL) and low-density lipoprotein (LDL). The result showed a significant ($p< 0.05$) decrease in total cholesterol with 25% (71.50 ± 9.95 g/L) and 50% (59.00 ± 13.70 g/L) cabbage supplement treated groups compared to the positive diabetic group (83.7 ± 8.25 g/L).

There was also a significant ($p < 0.05$) decrease in total triglyceride on all the treated groups with values of (36.87 ± 4.89 g/L; 75.00 ± 15.30 g/L and 32.22 ± 2.97 g/L) for both 10%, 25% and 50% cabbage supplementation compared to the positive control (163.40 ± 53,72 g/L).
However, there is a significant ($p < 0.05$) increase in serum high density lipoprotein in rats fed with 10% (39.59 ± 1.60 g/L) and 50% (65.12 ± 8.99 g/L) cabbage supplement when compared to the diabetic control (29.43 ± 5.17 g/L).

In addition, there was also a significant ($p < 0.05$) decrease in the serum low density lipoprotein at 10% (52.64 ± 5.50), 25% (45.68 ± 3.31) and 50% (30.93 ± 11.56) respectively as compared with the positive control (63.48 ± 9.71).

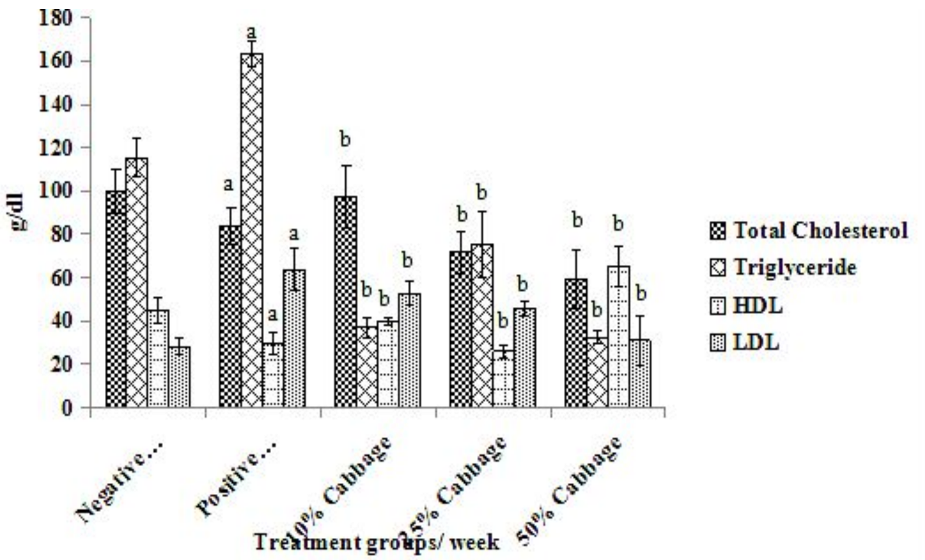


**Figure 2: The effect of cabbage supplement on serum total cholesterol, triglyceride, HDL and LDL on alloxan induced diabetic Wistar rat. [a]*P*< 0.05 vs. Negative control, [b]*P*< 0.05 vs. Positive control.**

Figure 3 depicts the results of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatise (ALP) in rats administered with different percentages of cabbage supplement. There was a significant increase ($p < 0.05$) in AST activity in all the treated groups 10%, 25% and 50% (25.22 ± 3.08; 44.95 ± 5.46 and 36.68 ± 4.82) as compared to the positive control ((13.62 ± 2.40).

In addition, there was also a significant increase ($p < 0.05$) in ALT activity in 10% (15.56 ± 2.82), 25% (35.35 ± 2.38) and 50% (28.6 ± 1.83) when compared with the positive control (6.13 ± 0.88).

Finally, there was also a significant decrease ($p < 0.05$) in rats treated with 10%, 25% and 50% (12.24 ± 0.71; 29.26 ± 5.95 and 15.41 ± 0.83) cabbage supplement compared with the positive control (28.88 ± 0.83).

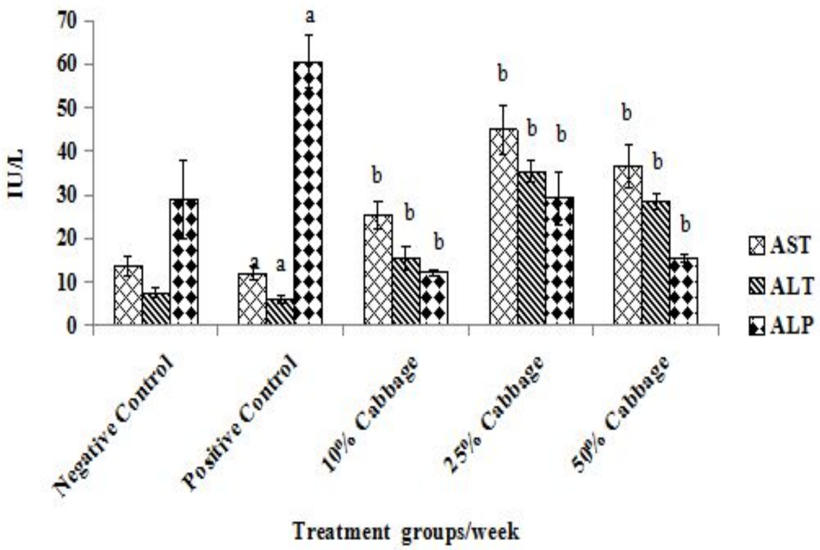


**Figure 3: The effect of cabbage supplement on serum AST, ALT and ALP on alloxan induced Wistar rats. [a]*P*< 0.05 vs. Negative control, [b]*P*< 0.05 vs. Positive control.**

## Discussion

Diabetes is a complex and complicated disease characterized by abnormalities in carbohydrate, protein, and lipid metabolism, causing a great challenge to physicians and other healthcare professionals who care for people with diabetes [27] [7]. Several studies have shown that vegetables play a protective role against the development of human diseases[28][29][30][31]. Vegetables like cabbage belonging to the cruciferous family exert a protective effect against many chronic degenerative diseases, including cancer [32] [33]. Glucosinolates are particularly abundant in cabbage and are believed to be the bioactive compound responsible for many of the biological effects attributed to them [32] [34]. However, cabbage is also an important source of other essential compounds such as polyphenols, carotenoids and phytosterols that exert an anti-inflammatory and antioxidant effect [35].

The observed decrease in serum levels of blood glucose in this study demonstrated the hypoglycaemic effect of cabbage supplement. These results agree with the work of [36] who demonstrated the hypoglycaemic effect of both extract of white and red cabbages in STZ induced type-2 diabetes in rats. The decrease may be due to the effect of different polyphenolic compound present in cabbage. These compounds, for example flavonoids and other alkaloids have been reported to have anti-diabetic properties. Our findings also agreed with that of [37] who demonstrated the hypoglycaemic and hypolipidaemic activities of red cabbage and manganese for the treatment of diabetes in rats. [38], also reported that the administration of anthocyanins (also found in cabbage) markedly decreased glucose levels and increase utilization of glucose by tissues in diabetic rats.

Lipid profile results showed abnormally high level of serum lipids in the diabetic subjects compared to the supplemented groups. The increase in lipid profile may be due to an increase in the mobilization of free fatty acids from the peripheral fat depots since insulin inhibits the hormone-sensitive lipase [39]. Since lipid abnormalities accompanied with premature atherosclerosis are the major causes of cardiovascular disease in patients with diabetes, ideal management for diabetes, should have a favourable effect on lipid profile [40].

The observed significant decrease in total triglycerol, total cholesterol, and LDL and an increase in the HDL level indicates that cabbage supplement have an antihyperlipidaemic effect. This result is in accordance with the findings of [41], who reported that ethanol extract of cabbage caused reduction in serum LDL, with increased HDL significantly. The glucose lowering action of the cabbage might be due to improved lipid metabolism apart from direct interaction with glucose homeostasis. The TG-lowering property could indirectly contribute to the overall antihyperglycemic activity through glucose-fatty acid cycle mechanism [42]. Epidemiological data as well as *in vitro* studies strongly suggest that cabbage having antioxidant phytochemical compounds have strong protective effects against major degenerative diseases including cardiovascular diseases, antihyperglycemic and hypocholesterolaemic [43] [44] [45].

The liver is regarded as the major organ of metabolism. The liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are considered as biomarkers of liver toxicity and are used in the evaluation of hepatic disorders [46].The result showed that serum level of ALT and AST in the cabbage supplement group were significantly higher than the control. While that of ALP was lower than that of control. These results is in accordance with the work of [36], who reported a significant increase in serum level of ALT and AST in diabetic rats treated with extract of both white and red cabbages in comparison with the control but in contrast with the ALP serum level result which also showed a significant increase. Our findings also disagree with the work of [37] [47], they observed a significant decrease in serum liver enzymes (ALT, AST and ALP) in diabetic rats who were treated with red cabbage powder and red cabbage extract. The increase in the activities of these serum enzymes may be due to the fact that cabbage when eaten in excess can be goitrogenic, and in goitrogenic conditions, liver enzymes activities are increased. This may explain the marked increase in the liver enzymes of AST and ALT.[48]

**Conclusion** The results obtained from the study demonstrated that cabbage supplementation alleviated hyperglycaemia and hyperlipidaemia associated with experimentally; induced diabetic Wistar rats. However, there is the need for more investigation on the toxicological profile of cabbage supplementation on the liver.

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