EFFECT OF WHITE CABBAGE (Brassica oleracea) AQUEOUS EXTRACT ON OXIDATIVE STRESS IN PRE-DIABETES – INDUCED MALE ALBINO RATS

Department of Nutrition and Dietetics, Ben S. Carson (Snr.) School of Medicine Babcock University, Ikeja Lagos

* Corresponding author’s email address: adeoyeb@babcock.edu.ng

ABSTRACT
Background: Oxidative stress affects serum insulin and induces insulin resistance which results in pre-diabetic condition.
Objective: This research investigated the effect of white cabbage aqueous extract on the serum insulin, insulin resistance and oxidative stress in pre-diabetes induced male albino rats.
Methods: Prepared extracts from fresh and dried cabbage and thirty male albino rats grouped into six were used for the study. The positive control was fed regular diet, while negative control was fed high sucrose feed and the four test groups were fed high sucrose feed and extract. Different test group received 50 or 100 mg/kg of either of the extracts. The fasting blood sugar at baseline and at two weeks’ interval was recorded. At the end of the experiment at six weeks, the serum insulin, oral glucose tolerance test and liver oxidative stress were determined. Results were analyzed using Analysis of Variance (p < 0.05) followed by Duncan Multiple Range Tests for blood glucose and Graph pad prism 5.
Results: The extracts controlled the serum insulin and cell-resistance to insulin of the rats. There was a reduction in the malondialdehyde (MDA) and significant reduction in the activities of superoxide dismutase (SOD) and catalase (CAT) by the extract while glutathione (GSH) increased. The MDA, SOD, CAT and GSH for the negative control group being 0.85 mg/ml, 0.8 mg/ml, 19.53 mg/ml and 5.18 µmol/ml while the highest for the test groups was; 0.84 mg/ml, 0.74 mg/ml, 17.37 mg/ml and 5.85 µmol/ml.
Conclusion: Cabbage had potential of reducing liver oxidative stress in pre-diabetic condition.
Keywords: Cabbage extract, blood sugar, hypoglycemic, oxidative stress

Introduction
In recent years, there has been profound interest in the role of oxidative stress in the precipitation of pre-diabetic condition (1) and there are reports of oxidative stress increasing in patients with diabetes mellitus (2). Oxidative stress results when reactive forms of oxygen are produced faster than they can be safely neutralized by the body antioxidant mechanisms (3). Various researches have shown that it affects the level of serum insulin and cause insulin resistance which are pathophysiologic determinants of pre-diabetic condition (1, 4, 5). The prevalence of pre-diabetes and diabetes imposes great burden on healthcare worldwide as patients also have increased risk of cardiovascular disease and other complications (6, 7). The estimated prevalence of high blood sugar (pre-diabetic condition) in Africa is 1% in rural areas, and ranges from 5% to 7% in urban sub-Saharan Africa and between 8-13% in more developed areas (8). The prevalence in Nigeria varies from 0.65% in rural Mugu (North) to 11% in urban Lagos (South) and data from the World Health Organization (9) suggests that Nigeria has the greatest number of people living with high blood sugar in Africa (8). The occurrence of a hypoglycaemic substance in plant materials and its concentration and ultimate purification as the hydrochloride of an unidentified compound, was reported by Dubin and Corbitt (10) which has led to many plant materials being researched for their potential to control blood sugar. Obatomi et al. (11) conducted a study on African mistletoe (Loranthusbengwensis L.) and hypoglycemic activity of Adansonia digitata extract was studied by Tanko et al. (12). Mohammed et al.(13)found the effect of aqueous leaves extract of Ocimum gratissimum on blood glucose levels of streptozocin induced diabetic Wistar rats while Abo et al. (14) surveyed plants from the Rutaceae, Leguminosae and Cucurbitaceae families commonly used by traditional healers in South West Nigeria for the treatment of diabetes mellitus. Furthermore, Ogundipe et al. (15) determined the hypoglycemic potentials of methanolic extracts of selected plant foods; H. sabdarifff, A. occidentalis, S. americanum, V. amygdalina, G. latifolium, H. sabdariffa, A. occidentalis and S. americanumin alloxanized mice. Nwanjo, (16) showed that the aqueous extract of V. amygdalina leaves have both hypolipidaemic and antioxidant properties in diabetic rat models. Presently, several species of medicinal plants used for the management of diabetes mellitus worldwide have been evaluated by Ediriweera and Rathnasooriya (17). Cabbage (Brassica oleracea var. Capitata L.) belongs to the Brassicaceae family and is closely related to the broccoli, cauliflower and brussels...
The samples were obtained from Babcock University, Ilishan–Remo Ogun State, Nigeria, and identified at the Botany Department, Federal University of Agriculture Abeokuta with herbarium number 028. To prepare extract from the dried cabbage, the cabbage outer layer was removed and the remaining part cut into small pieces after washing. The cabbage pieces were weighed and dried in an oven at 40°C and ground using a laboratory grinder (23). The cabbage (125g) was extracted with water in five conical flasks (25g per flask) in a continuous shaker water bath at 40°C for 48 h (24) and filtered.

For extract from fresh cabbage, the outer part of the cabbage was removed, washed, weighed, cut into pieces and blended. The blended cabbage was sieved using a 0.03mm diameter sieve to obtain extract. The extracts from the dried and fresh cabbage were poured into glass petri dishes and placed in the hot air oven at 40°C for a day to dry allowing for the removal of moisture. The dried extract was then homogenized into a fine powder using an agate mortar and pestle and stored in a refrigerator for subsequent studies.

Ethical approval
Ethical approval was obtained from Babcock University Health Research Ethics Committee (NHREC/17/12/2013) before the commencement of the research.

Materials and methods
Extraction of cabbage constituents
Cabbage (white spp.) was obtained from Eleweran vegetable market Abeokuta, Nigeria and identified at the Botany Department, Federal University of Agriculture Abeokuta with herbarium number 028. To prepare extract from the dried cabbage, the cabbage outer layer was removed and the remaining part cut into small pieces after washing. The cabbage pieces were weighed and dried in an oven at 40°C and ground using a laboratory grinder (23). The cabbage (125g) was extracted with water in five conical flasks (25g per flask) in a continuous shaker water bath at 40°C for 48 h (24) and filtered.

For extract from fresh cabbage, the outer part of the cabbage was removed, washed, weighed, cut into pieces and blended. The blended cabbage was sieved using a 0.03mm diameter sieve to obtain extract. The extracts from the dried and fresh cabbage were poured into glass petri dishes and placed in the hot air oven at 40°C to dry after which they were kept in the refrigerator for subsequent studies.

Animal studies
The study was carried out at the Animal Facility Babcock University, Ilishan – Remo Ogun State with 30 male albino rats weighing 110-120g which were obtained from Babcock University Animal Facility. The rats which were 15-18 weeks old were housed in cages at a temperature of 28±2 °C for 10 days before the experiment, allowed free access to regular rat pellet (25g) and were randomly divided into six groups (5 per group) of two control groups (positive control A1 and negative control A2) and four test groups (B1, B2, B3 and B4). The group A1 was placed on commercial regular rat feed while the group A2 was fed only high sucrose (30% concentration) feed to induce type 2 pre-diabetic condition (26, 27). The test groups were fed high sucrose feed alongside cabbage extract for six weeks. Group B1 received 50 mg/bodyweight of cabbage fresh extract, group B2 received 100 mg/bodyweight of cabbage fresh extract, group B3 received 50 mg/bodyweight of cabbage dried extract and group B4 was given 100mg/bodyweight of cabbage dried extract. The extracts were administered orally in the morning with the aid of a syringe while water and food were given morning and evening during the experiment. Fasting blood sugar was determined at the beginning (baseline) and every two weeks during the period of the experiment using glucometer. Twenty-four hours after administration of the last dose of the extract, animals were fasted and blood samples were collected from the tail vein for determination of oral glucose tolerance test and blood samples were also obtained from the eye (orbital plexus blood samples) for estimation of serum insulin. In order to collect the liver samples, the rats were made unconscious by means of cervical dislocation. The liver samples were homogenized in deproteinizing solution for determination of oxidative stress. The rats died as a result of the cervical dislocation to remove their liver and were disposed of by putting them in a garbage bag and burying in the ground.

Chemical analysis
Determination of serum insulin
All reagents and rats’ liver samples (stored below 0°C) were brought to room temperature before use and ELISA (enzyme-linked immunosorbent assay) was used for the analysis. Reagents used for ELISA procedure includes; Insulin calibrator (blank and reference), Biotinylated Detection Ab (Avidin-biotin) working solution, HRP (Horseradish Peroxidase) conjugate working solution, substrate solution (H₂O₂-TMB 0.26 g/L), stop solution (Sulphuric acid 0.15 mol/L) and wash solution (NaCl 45 g/L; Tween- 20 55 g/L). The samples were centrifuged at 3,000 revolutions for 15 minutes and again after thawing before the assay. All the reagents were mixed thoroughly by gently swirling to avoid foaming before pipetting. All samples and standards were assayed in duplicate. A 100µL of standard, blank, or sample was added per well. Added to the blank well were the reference standard and sample diluents. Solutions were added to the bottom of micro ELISA plate well, wall touching and foaming was avoided as possible. This was mixed gently and the plate covered with the sealer. Incubation was done for 90 minutes at 37°C. The liquid of each well was removed (not washed). Immediately 100µL of Biotinylated Detection Abworking solution was added to each well and covered with the plate sealer. The plate was gently tapped to ensure thorough mixing and incubation was for 1 h at 37 °C. Each well was aspirated and washed, repeating the process three times. Washing was done by filling each well with wash buffer approximately
350 µL using a squirt bottle, complete removal of liquid at each step was ensured. After the last wash, the remaining wash buffer was removed by aspirating or decanting. The plates were inverted and patted against thick clean absorbent paper.

Then 100µL of HRP conjugate working solution was added to each well, covered with the plate sealer, incubated for 30 min at 37°C and wash process was repeated for five times. Also, 90 µL of substrate solution was added to each well and covered with a new plate sealer. The plate was incubated for about 15 min at 37°C and the plate was protected from light. The reaction was terminated, when apparent gradient appeared in standard wells.

Then finally, 50 µL of stop solution was added to each well and there was formation of yellow colouration immediately. The optical density (OD value) of each well was determined at once, using a micro-plate reader set to 450 nm (28).

**Determination of oral glucose tolerance test**

The test was determined according to the method described by Hedrich (29). Rats were fasted overnight (14 h), placed in fresh cages without food, the cages and the rats were identified and the weight of each rat was recorded to within 0.1g. The volume of 20% glucose solution required for oral gavage of 2 g glucose/kg at an oral injection volume of 10 µl/g body weight was calculated for each rat and recorded. Experiment record sheet, strips for glucose measurement, and a 1 mlsyringe for each animal containing the calculated volume of glucose to be gavaged were prepared. The blood glucose monitor was calibrated with the standard strip. A small (~3µl) blood sample was placed directly on to the test strip and inserted into the blood glucose monitor. Direct pressure was applied to the incision until the blood clot and the rat was returned to its cage. After all the rats have been measured for basal glucose concentrations, the glucose solution was administered by oral gavage to each animal at 30 seconds interval between animals and the timer started with the first rat gavage. All injections were finished within 15 minutes in order to perform the next blood glucose measurement at T = 30 min. At T = 30 min, the blood glucose was measured again, starting with the first rat gavage and using the same time interval used for gavage, until all the rats in the cohort have been measured. The timing was followed as closely as possible because it is very important. To restart the bleeding, the clot from the first incision was removed and the tail massaged if blood flow is inadequate. This was repeated at T= 60 and 120 min after gavage. At the end of the experiment, the rats were returned to their original cages and provided plenty of food and water. It was ensured that no animal was bleeding excessively.

**Determination of liver enzyme activity**

Superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) are the main endogenous enzymatic defense systems of all aerobic cells. They give protection by directly scavenging superoxide radicals and hydrogen peroxide, converting them to less reactive species. SOD catalyzes the dismutation of superoxide radical (O$_2^\cdot$) to hydrogen peroxide (H$_2$O$_2$). The CAT and GSH are also important part of enzymatic defense and neutralize H$_2$O$_2$ into H$_2$O (30). The hepatic superoxide dismutase (SOD) activity, hepatic catalase (CAT) activity and hepatic reduced glutathione (GSH) level were determined by the method described by Ismail et al. (31) and Ismail et al. (32). While assessment of lipid peroxidation was determined using the procedure of Vashney and Kale (33) and expressed as micromolar of malondialdehyde (MDA)/g tissue.

**Statistical analysis**

Analysis of variance (ANOVA) was used to determine significant difference (p < 0.05) in the blood glucose of the test and the control groups and Duncan multiple range test used to separate the means using SPSS version 20.0 while results of serum insulin, oral glucose tolerance test and liver oxidative stress were analysed using Graph pad prism 5.

**Results**

Blood glucose of the control groups and the test groups is shown in Table 1. There was a significant difference in the blood glucose at baseline with the blood glucose of A2 group being the highest (106.33±14.57 mg/dl) while the A1 group had the lowest (87.25 ± 7.89 mg/dl). Blood glucose of the test groups ranged from 96.00 ± 5.35 mg/dl to 102.75 ± 10.28 mg/dl. At fourth week, blood glucose of the A2 group was lowered (48.67 ± 16.26 mg/dl) to that of other groups while at sixth week it increased (62.00 ± 12.00 mg/dl) more than that of the other groups.
Table 1: Blood glucose (mg/dl) of the control and test groups over six weeks

<table>
<thead>
<tr>
<th>Groups</th>
<th>Baseline</th>
<th>2 weeks</th>
<th>4 weeks</th>
<th>6 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>87.25 ± 7.89b</td>
<td>87.80 ± 5.68b</td>
<td>65.75 ± 6.24a</td>
<td>44.00 ± 9.42bc</td>
</tr>
<tr>
<td>A2</td>
<td>106.33 ± 14.57a</td>
<td>53.75 ± 11.15a</td>
<td>48.67 ± 16.26a</td>
<td>62.00 ± 12.00a</td>
</tr>
<tr>
<td>B1</td>
<td>96.00 ± 5.35ab</td>
<td>70.00 ±11.36ab</td>
<td>64.25 ± 11.09a</td>
<td>43.25 ± 3.30bc</td>
</tr>
<tr>
<td>B2</td>
<td>102.75 ± 10.28a</td>
<td>64.50 ± 7.33ab</td>
<td>59.75 ± 10.81a</td>
<td>55.50 ±11.73ab</td>
</tr>
<tr>
<td>B3</td>
<td>100.00 ± 8.49ab</td>
<td>57.50 ± 6.46a</td>
<td>59.50 ± 3.54a</td>
<td>35.50 ± 24.75c</td>
</tr>
<tr>
<td>B4</td>
<td>100.00 ± 7.94ab</td>
<td>68.00 ± 8.49a</td>
<td>54.33 ± 6.35c</td>
<td>47.00 ± 2.65bc</td>
</tr>
</tbody>
</table>

Means ± SD of duplicate determinations, values in the same column with the same superscript are not significantly different (p ≤ 0.05), A1 = Positive control, A2 = Negative control, B1 = Group fed 50 mg/kg bodyweight, fresh cabbage extract, B2 = Group fed 100mg/kg bodyweight fresh cabbage extract, B3 = Group fed 50 mg/kg bodyweight dried cabbage extract, B4 = Group fed 100 mg/kg bodyweight dried cabbage extract.

The serum insulin of the test and control groups is presented in Fig. 1. There was no significant difference in the serum insulin of the control and test groups. The group B2 had the highest (70pmol/L) serum insulin followed by the A1 (67pmol/L). The group A2 had the same level of serum insulin (53pmol/L) with the group B4.

![Fig. 1: Level of serum insulin (pmol/L) of the control and the test groups](image)

At baseline, the blood glucose (43.25±1.41mg/dl) of the group B1 (Fig. 2) while the A2 group had the highest blood (62.0±5.12mg/dl). At 30 min the group B2 (194.75±24.5 mg/dl) and at 60 min that of the group A2 become higher (187.33± 55.4 mg/dl) while the blood glucose of other groups was lowered.
Fig. 2: Blood glucose level of the control and the test groups during the oral glucose tolerance test
A1 = Positive control, A2 = Negative control, B1 = Group fed 50 mg/kg bodyweight, fresh cabbage extract, B2 = Group fed 100 mg/kg bodyweight fresh cabbage extract, B3 = Group fed 50 mg/kg bodyweight dried cabbage extract, B4 = Group fed 100 mg/kg bodyweight dried cabbage extract

Lipid peroxidation determined as MDA concentration (mg/ml) of the control and test rats is presented in Fig. 3. The MDA concentration of the negative control group was $0.85 \pm 0.26$ mg/ml and the least $(0.62 \pm 0.06$ mg/ml) was that of the group administered 100mg/kg of fresh cabbage extract. The MDA of the test groups were significantly reduced compared to the control negative.
Fig. 3: MDA. Concentration (Lipid peroxidation) of the control and the test groups
A1 = Positive control, A2 = Negative control, B1 = Group fed 50 mg/kg bodyweight fresh cabbage extract, B2 = Group fed 100 mg/kg bodyweight fresh cabbage extract, B3 = Group fed 50 mg/kg bodyweight dried cabbage extract, B4 = Group fed 100 mg/kg bodyweight dried cabbage extract; *= p < 0.05 when comparing with the A1 group; # = p < 0.05 when comparing with the A2 group

In Fig. 4, there was reduction in the superoxide dismutase (SOD) activity of groups administered cabbage extract with significant reduction (0.59 ± 0.08 and 0.54 ± 0.02 mg/ml) in the groups B3 and B4 while the SOD of the A1 group was the highest (0.82 ± 0.10). There was also a reduction in the catalase (CAT) activity of the test groups with significant reduction in the groups B1 (11.72 ± 3.68 mg/ml) and B4 (12.18 ± 1.01 mg/ml) while the CAT of the A2 group was the highest (19.53 ± 2.12 mg/ml) (Fig. 5).

Fig. 4: Superoxide dismutase activity of the control and the test groups.
A1 = Positive control, A2 = Negative control, B1 = Group fed 50 mg/kg bodyweight, fresh cabbage extract, B2 = Group fed 100 mg/kg bodyweight fresh cabbage extract, B3 = Group fed 50 mg/kg bodyweight dried cabbage extract, B4 = Group fed 100 mg/kg bodyweight dried cabbage extract; *= p < 0.05 when comparing with the A1 group; # = p < 0.05 when comparing with the A2 group
Fig 5: Catalase activity of the control groups and the chemo preventive test groups.

A1 = Positive control, A2 = Negative control, B1 = Group fed 50 mg/kg bodyweight, fresh cabbage extract, B2 = Group fed 100 mg/kg bodyweight fresh cabbage extract, B3 = Group fed 50 mg/kg bodyweight dried cabbage extract, B4 = Group fed 100 mg/kg bodyweight dried cabbage extract; * = p < 0.05 when comparing with the A1 group; # = p < 0.05 when comparing with the A2 group

Glutathione level of the control and test groups are presented in Fig. 6. The glutathione level of the groups administered 100 mg/ml of fresh cabbage (5.65 ± 0.21 µmol/ml), and 100 mg/ml of dried cabbage extract (5.85 ± 0.24 µmol/ml) were higher than that of the positive (5.15 ± 0.45 µmol/ml) and negative (5.18 ± 0.46 µmol/ml) control groups.

Fig 6: Glutathione level of the control and the test groups

A1 = Positive control, A2 = Negative control, B1 = Group fed 50 mg/kg bodyweight, fresh cabbage extract, B2 = Group fed 100 mg/kg bodyweight fresh cabbage extract, B3 = Group fed 50 mg/kg bodyweight dried cabbage extract, B4 = Group fed 100 mg/kg bodyweight dried cabbage extract; * = p < 0.05 when comparing with the A1 group; # = p < 0.05 when comparing with the A2 group.
Discusison
There was a reduction in the blood sugar of all the animals in the different group from second week and this could be attributed to low feed intake as a result of the rats not being familiar with the feed. The positive control group which was also in proximity was also influenced. However, it was found that the blood glucose of the rats in A2 group increased at sixth compared with that of the A1 and test groups which was an indication of the efficacy of the extracts given. This observation supported earlier report of Amnah (21) who found that red cabbage has hypoglycaemic effect in diabetic rats. It was found that the high rise in blood sugar commonly observed in pre-diabetics could only be achieved with longer period of feeding the rats with the high sucrose feed which was a limitation that should be mentioned in this study.

However, the results of serum insulin and the oral glucose tolerance test showed the on-set of the pre-diabetic condition. Major pathophysiology in pre-diabetes is decline in insulin secretion and resistance to insulin (4, 34). It was found that the positive control group on regular diet and the test groups (except for the group B4) had higher level of insulin than the A2 group. Thus, there might have been a decline in the insulin production of the A2 group which was on high sucrose feed and was not subjected to any treatment. The low serum insulin of the A2 group was likely to have been the cause of the higher blood sugar recorded by this group. The cabbage extracts administered had positive influence on the beta-cells of the pancreas such that the blood sugar and serum insulin of the A1 group was within the range for the test groups. These findings are been corroborated by the report of Wang and Wang (35) who suggested that antioxidants has potential benefits as a therapeutic method for type 2 diabetes in respect of improving beta cell regeneration.

From the study it was found that while the test groups and the positive control attained their peak at 30 minutes during oral glucose tolerance test, the negative control group did not attain its peak until 60 minutes when the blood glucose of the other groups have already been significantly lowered. Its peak (187.33±5.54 mg/ml) was higher than the peak for other groups, an indication of higher resistance to insulin which supported earlier report that high sucrose feed induced insulin resistance in rats (36). However, the results showed that white cabbage aqueous extract had capability of reducing insulin resistance in hyperglycemic condition which was corroborated by the report of Attanayake et al. (37) on antihyperglycaemic activity of medicinal plant.

Some diseases of the liver are associated with an increase or decrease in anti-oxidant’s contents (38, 39). Usually hepatic antioxidants increase at the beginning of hepatic disease and decrease in severe hepatic injury. The result of lipid peroxidation showed increased concentration of MDA in the negative control group while there was reduction in the level of MDA of the test groups B1, B2 and B4 while the B3 group seemed to have poor control of the MDA. This finding was in agreement with the findings of Amnah (21) on effect of red cabbage on MDA. The extracts from the fresh and dried cabbage exerted significant effect on the SOD and CAT by lowering the levels when compared to the negative control.

High level of antioxidant enzymes (SOD and CAT) in the A2 group might be an indication of precipitation of liver disease (mild hepatic problem) while the level of activity of these enzymes in the test groups might portray the ability of the extract to oppose the precipitation of liver disease. This observation was supported by earlier reports of Kataya and Hamza (20), Ercin et al. (40), Broide et al. (41) and Ismail et al. (21). Contrary to these reports, GSH increased in the test groups when compared with the A2 group except for the B1 group. The100mg/kg body weight of both extracts seemed to favour increase in the GSH activity which also function to prevent accumulation of superoxide and hydroxyl radicals in cell so as to prevent cell damage (26).

Conclusion
The potential of white cabbage in helping to maintain the level of serum insulin, prevent insulin resistance and ameliorate oxidative stress in pre-diabetic condition was demonstrated in this study.

References


