

Anacardium Occidentale leaves extract Regulate Cholesterol and Demonstrate Anti-diabetics effect on Wistar Rats (*Rattus Novergicus*)

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Abstract

Diabetes mellitus is the most common metabolic disorder, characterized by hyperglycemia that results from an absolute or relative insulin deficiency and is associated with long-term complications affecting the eyes, kidneys, heart and nerves. This research work investigates the hypoglycemic and hypolipidemic effects of Anacardium Occidentale leaf extract on alloxan induced Diabetic Wistar Rats. Rats were made diabetic by intraperitoneal injection of 120 mg/kg body weight of alloxan monohydrate. Rats of both sexes were divided into 5 groups (n=5). Group 1 (control), group 2 (treated with glibenclamide 5 mg/kg; orally), group 3-low dose (treated with 150 mg/kg body weight of the extract; orally), group 4-high dose (treated with 300 mg/kg body weight of the extract; orally) and group 5 (diabetes untreated). The administration was done once daily orogastrically, during which blood glucose level was monitored at 3 days interval for a period of 2 weeks with the aid of Glucometer. Animals were sacrificed 24 hours after the last administration by cervical dislocation. Blood sample was collected from the eye for estimation of lipid profile. Induction of alloxan resulted in significant increase in glucose, total cholesterol, triglyceride, and low density lipoprotein levels in the rats. The administered extract decreased the concentration of total cholesterol, low density lipoprotein and triglyceride to an extent and increased the concentration of high density lipoprotein. This result suggests the hypoglycemic and antioxidant effect of the extract of *A. occidentale* in alloxan-induced diabetic rats and supports the traditional herbal drugs for the treatment of diabetes mellitus.

Keywords: Anacardium Occidentale, Antioxidant, Alloxan, Diabetes, Lipid profile

INTRODUCTION

Diabetic complications have shown increased oxidative stress and development of complications through the metabolism of excessive glucose and free fatty acids in diabetic and insulin-resistant states. Diabetes is a chronic metabolic disorder that continues to present as a major health problem worldwide. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action and is associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism (Haffner et al., 1998). Diabetes mellitus is classified into two types, insulin dependent diabetes mellitus (IDDM, Type 1) and non-insulin-dependent diabetes mellitus (NIDDM, Type II). Type I diabetes mellitus is an autoimmune disease characterized by a local inflammatory reaction in and around islets that is followed by selective destruction of insulin-secreting cells (Haffner et al., 1998; Windler, 2005). Type II diabetes is characterized by peripheral insulin resistance and impaired insulin secretion. Type II diabetes often exhibits

an atherogenic lipid profile, which greatly increases their risk of Cardio-vascular diseases CVD when compared with people without diabetes (Haffner et al., 1998; Windler, 2005). Insulin affects many sites of mammalian lipid metabolism (Jain and Gupta, 1980; Agrawal et al., 1985). It stimulates synthesis of fatty acid in liver adipose tissue and in the intestine. The insulin has also been reported to increase the cholesterol synthesis. The activity of lipoprotein lipase in white adipose is also increased. An early intervention to normalize circulating lipids has been shown to reduce cardiovascular complications and mortality. The consumption of a variety of local herbs by man is believed to contribute significantly to the improvement of human health in terms of prevention, and or cure or treatment of diseases because plants have long served as a useful and natural source of the therapeutic agents (Roberts and Tyler, 1997). Some of these local herbs may include *Anacardium occidentale*. Extracts from roots, leaves, stems and fruits of *A. occidentale* L., (Sokeng et al., 2001) have been used reported to display hypoglycemic effects in folk medicine, but only few of them have been investigated (Miura et al., 2002). It also contains some minerals which include Sodium, Nitrogen, Potassium, Calcium, Magnesium, Iron, phosphorus, Copper and selenium. Phytochemical screening was performed on young cashew leaves, the screening revealed the presence of carbohydrate, alkaloids, tannins, flavonoids, phenols, oxalate and saponins either in High (+++), moderate (++) and low (+) concentrations (George, 2000). Indications from the results depicted usefulness of the young cashew leaves in the treatment of some common diseases like pile, malaria fever, asthma, dysentery, dermatitis etc (Ilonzo, 1995).

Therefore, this study aimed at investigating the hypoglycemic effects and corresponding changes in the Lipid profile following the administration of *Anacardium occidentale* on Alloxan Induced diabetes in Adult wistar rats

MATERIAL AND METHODS

Preparation of ethanolic extract

The leaves of *A. occidentale* were collected from Bingham University Karu, Nasarawa State. Botanical identification and authentication were performed at the National Institute for pharmaceutical research and development Idu, Abuja. Herbarium voucher specimen number NIPRD/H/6581 was collected by Dr. Jemilat A Ibrahim. Leaves were cleaned with tap water, dried at room temperature for 2 weeks and ground into powder. 270g of plant powder sample was extracted in ethanol 2.3L (2300ml) by maceration method for 24 hours. The macerated sample was filtered using muslin cloth at room temperature. The filtrate was concentrated using rotary evaporator and finally dried on the water bath in an evaporating dish. The dried extract was weighed to be 49.1g yielding 17.0% (James, 1983).

Table 1. Phytochemical screening of *Anacardium Occidentale*

| Group | Test | Inference | |
|---------------|--------------------------------------|-------------------|-----------------|
| | | Ethanolic extract | Powdered sample |
| Tannins | Ferric chloride test | + | + |
| Saponins | Froth test | - | - |
| Alkaloids | Dragendoff reagent Wagner's | + | + |
| Terpenes | Liebermann-Burchard | - | - |
| Sterols | Salkowski's | - | - |
| Glycosides | Keller-Killiani test for deoxy sugar | + | + |
| Phenols | Ceric ammonium nitrate | + | + |
| Resins | Copper acetate | + | + |
| Flavonoids | Lead acetate NaOH | + | + |
| Carbohydrates | General test-Molisch's test | + | + |

Key

Positive (+) = present

Negative (-) = absent

Animal source and handling

Wistar Rats of both sexes weighing between 150-200 g were used in this study. Animals were divided into five (5) groups of seven (7) Rats, the rats were housed and raised in the Animal House of the Faculty of Health Science, Bingham University, Karu, Nigeria with a 12 h light and 12 h dark cycle. The animals were kept in the experimental animal room for 2 weeks for acclimatization and fed with free standard pellet diet and tap water

Induction of diabetes using alloxan

After fasting for 18 hours, the rats were intraperitoneally injected with freshly prepared alloxan monohydrate solution (120 mg/kg), 0.76g of alloxan was weighed individually for each animal according to the body weight and then dissolved in 25ml of distilled water and administered within few minutes of its preparation. A day after induction, blood glucose level increased and was detected by using commercially available kit (Accu-Chek Active Test Meter) and rats showing hyperglycemia with blood glucose >200 mg/dl 24 hours after alloxan monohydrate injection were selected for the experiments (Abdel *et al.*, 1997; Mukherjee *et al.*, 2006).

Experiment

Group 1 Control (The animals were fed with feed and water), Group 2 - Diabetes treated (The animals were treated with standard drugs; glibenclamide (5mg/kg) (Trivedi *et al.*, 2004). Group 3-diabetes treated (The animals were treated with low dose of extract 150mg/kg) (Leonard *et al.*, 2006). Group 4 - diabetes treated (The animals were treated with high dose of extract 300mg/kg) (Leonard *et al.*, 2006). Group 5 - diabetes untreated (The animals were induced with diabetes using 120mg/kg b/w of alloxan (Kiran *et al.*, 2011) without administering the extract of young leaves of *A. occidentale*. All the treatments were given once a day orally for sixteen days (16) (Lawrence *et al.*, 2005). During the study, standard food and water were made freely available to animals. The fasting blood glucose levels were determined at intervals of 0, 1, 4, 7, 10, 13, and 16 days. On day 0 there were no significant differences in the fasting blood glucose levels in all the groups when compared with the control. Blood glucose level was detected by using commercially available kit (Accu-Chek Active Test Meter).

Determination of fasting blood glucose and lipid profile

To determine the blood glucose level, all animals had overnight fasting. All blood samples were collected from the tail artery of the rats at interval of 0, 1, 4, 7, 10, 13, 16 days. Blood glucose was estimated by using glucometer (Accu-Chek sensor from Roche Diagnostic Corporation) and results were expressed as mg/dl (Asha *et al.*, 2011). Serum levels of low-density lipoprotein-cholesterol (LDL-C) was calculated by the equation: $LDL = TC - HDL - TG/2.2$, high density lipoprotein (HDL) was estimated using accurex kit precipitation method, total cholesterol (TC) was estimated using accurex diagnostic kit cholesterol oxidase peroxidase method (reference range 3.5-6.0) and triglyceride (TG) was also estimated using accurex diagnostic kit (reference range 0.50-1.76).

Statistical analysis

Statistical analysis was performed using student's t-test. Values were represented as mean \pm SD. Data was analysed using analysis of variance and group means and significant difference accepted at $p < 0.05$. SPSS was used for analysis and Microsoft excel 2010 was used for the production of charts.

RESULTS

Table 1. Phytochemical screening of *Anacardium Occidentale*

| Groups | DAY 0 | DAY 1 | DAY 4 | DAY 7 | DAY 10 | DAY 13 | DAY 16 |
|---------------|------------------|------------------|-------------------|-------------------|-------------------|------------------|-----------------|
| Control | 111.0 \pm 2.99 | 111.4 \pm 3.52 | 108.4 \pm 2.52 | 101.43 \pm 1.53 | 101.4 \pm 1.52 | 101.4 \pm 1.52 | 61.0 \pm 10.1 |
| Glibenclamide | 93.6 \pm 10.66 | 333 \pm 2 | *440.9 \pm 79.1 | *326.6 \pm 4.05 | *255.7 \pm 6.54 | 113.7 \pm 40.2 | 28.4 \pm 7.7 |
| Low Dose | 105 \pm 5.36 | 421.4 \pm 45.9 | 373.3 \pm 766.6 | *281.7 \pm 54.6 | *183.7 \pm 2.73 | *65.6 \pm 18.5 | 68.6 \pm 31.6 |
| High Dose | 109.4 \pm 4.99 | 435.3 \pm 85.5 | 277.7 \pm 53.2 | *172.6 \pm 66.4 | 171.9 \pm 46.5 | 92.7 \pm 17.3 | 82.1 \pm 19.1 |

Mean \pm S.E.M; significantly different from control, $p < 0.05$

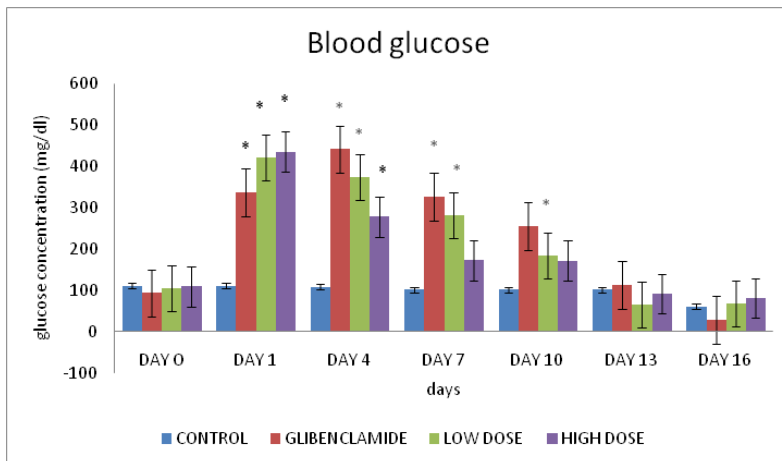


Figure 1. Concentration of blood glucose level

*significantly different at p<0.05

Table 2. Effect of ethanolic extract of *A. occidentale* (young leaves) on lipid profile of alloxan induced diabetic rats

| | Total cholesterol | Triglyceride | High density Lipoprotein | Low density Lipoprotein |
|---------------|-------------------|--------------|--------------------------|-------------------------|
| Control | 50.8±4.11 | 149.7±4.85 | 20.4±1.86 | 7.97±1.78 |
| Glibenclamide | 44.4±10.0 | 136±24.1 | 34.7±18.3 | 11.8±4.76 |
| Low dose | 38.1±7.22 | 136±229 | 17.3±3.43 | 9.1±3.74 |
| High dose | 44.4±9.08 | 132.6±23.3 | 15.3±2.88* | 15.3±2.88 |

Mean ± S.E.M; significantly different from control, p<0.05

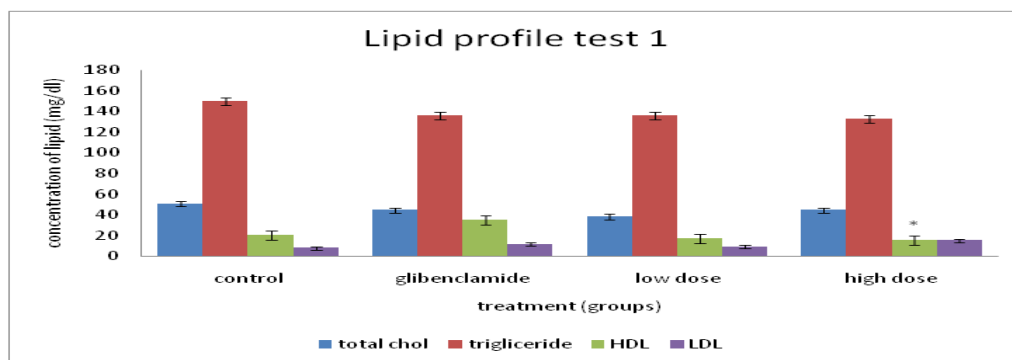


Figure 2. Lipid profile test 1

*significantly different at p<0.05

HDL – High density lipoprotein

LDL – Low density lipoprotein

Table 2: Effect of ethanolic extract of *A. occidentale* (young leaves) on lipid profile of alloxan- induced diabetic rats.

LIPID PROFILE TEST 2

| | Total cholesterol | Triglyceride | High density Lipoprotein | Low density Lipoprotein |
|---------------|-------------------|--------------|--------------------------|-------------------------|
| Control | 14.4±4.21 | 147.4±4.57 | 16.3±1.31 | 9.66±1.81 |
| Glibenclamide | 39.7±10.5 | 104±26.9 | 13.2±3.63 | 6.86±2.34 |
| Low dose | 30.2±9.9 | 101.7±26.3 | 11.2±3.06 | 10.1±4.23 |
| High dose | 34.9±6.1 | 122.3±20.6 | 14.3±2.69 | 4.21±1.40 |

Mean ± S.E.M; significantly different from control, p<0.05

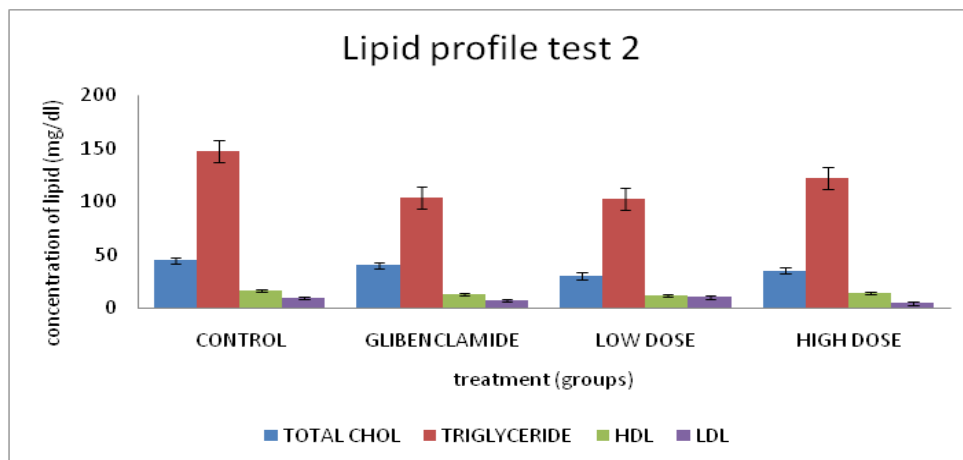


Figure 3. Lipid profile test 2

*significantly different at $p < 0.05$
 HDL – High density lipoprotein
 LDL – Low density lipoprotein

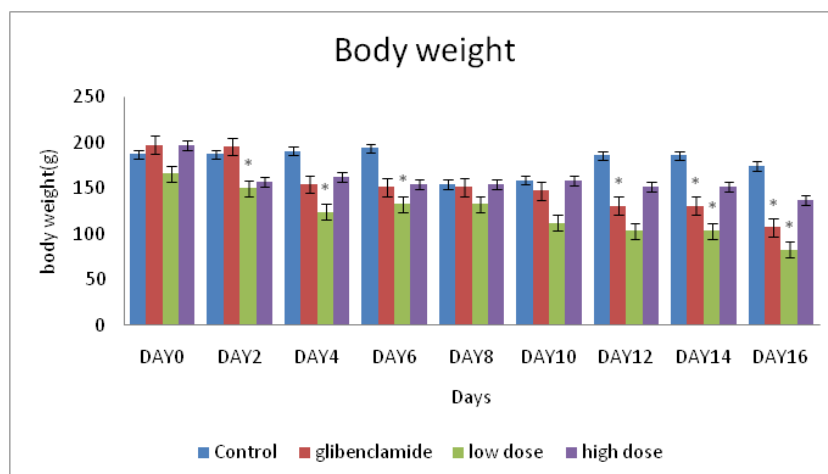


Figure 4. Body weight of rats

*significantly different at $p < 0.05$

DISCUSSION

Insulin produced by beta cells of the pancreas is the principal hormone that regulates uptake of glucose from the blood. Therefore deficiency of insulin or the insensitivity of its receptor plays a central role in all forms of diabetes mellitus. Alloxan is a urea derivative which causes selective necrosis of the β (Beta)-cells of pancreatic islets (Etuk, 2010; Iranloye *et al.*, 2011). As it has been widely accepted that alloxan selectively destroys the insulin-producing beta-cells found in the pancreas, hence it is used to induce diabetes in laboratory animals. The toxic action of alloxan on pancreatic beta cells involve oxidation of essential sulphhydryl(-SH groups), inhibition of glucokinase enzyme, generation of free radicals and disturbances in intracellular calcium homeostasis (Dunn *et al.*, 1943; Szkudelsk *et al.*, 2001; Dhanesha *et al.*, 2012).

The phytochemical screening done showed that the plant contains tannins, alkaloids, glycosides, phenols, resins and carbohydrate which is in agreement with the previous report of Dietewa, 2004 and as a result, these constituents significantly reduced blood glucose. Classes of chemical compounds isolated from plants are documented to have the potential to decrease the blood glucose level (Oubre *et al.*, 1997; Ragavan and Krishnakumari, 2006). Thus, the significant anti diabetic effect of extracts of *A. occidentale* could be due to the possible presence of the aforementioned

constituents in the part of the plant used in this particular study which could act synergistically or independently enhancing the activity of glycolytic and glyconeogenic enzymes.

After determining the blood glucose level, it showed that there was significant increase in blood glucose level in glibenclamide group, low dose group and high dose group 24 hours immediately after the induction of alloxan. On the 4th day, blood glucose level of glibenclamide also increased significantly compared to control. This may be as a result of the mechanism of alloxan in which experimental studies have demonstrated that alloxan evokes a sudden rise in insulin secretion in the presence or absence of glucose which appears just after alloxan treatment (Szkudelski *et al.*, 1998; Lachin and Reza, 2012). This particular alloxan-induced insulin release occurs for short duration followed by the complete suppression of the islet response to glucose even when high concentrations of glucose were used. Still on the 4th day, blood glucose level of low dose and high dose groups were significantly decreased ($p < 0.05$) compared to control. On the 7th day, the concentration of blood glucose statistically significantly decreased ($p < 0.05$) in glibenclamide and low dose group compared to control. The concentration of blood glucose also decreased in high dose group but it was not significant, this might be connected with increased availability of antioxidants that are important components and co – factors of the antioxidant enzymes. On the 10th day, there was significant decrease in blood glucose level in low dose group, the decrease was also seen in glibenclamide group and high dose group although not statistically significant. The decrease in blood glucose level was consistent till the 13th and 16th day of administration of ethanolic extract but it was not significant. A closer look revealed significant hypoglycemic effect of the ethanolic extract since it lowered the blood glucose to nearly half the normal value of alloxan induced diabetic rats at ($p < 0.05$) (Sanchez *et al.*, 2000). It was observed that the high dose was more consistent and effective in reducing blood glucose level than glibenclamide and low dose from the 4th day of administration to the 16th day, followed by low dose which also decreased but decreased drastically on the 16th day when compared to high dose and glibenclamide. Glibenclamide showed the least level of reduction when compared to low dose and high dose group, this may be as a result of its mechanism of action which works by inhibiting the sulfonylurea receptor 1 (SUR1), the regulatory subunit of the ATP-sensitive potassium channels (Serrano *et al.*, 2006), this inhibition causes cell membrane depolarization opening voltage-dependent calcium channel.

Factors such as age, sex, and genetics influence lipid profile. Certain aspects of lifestyle, including diet, level of physical activity, level of diabetes control, and smoking status, also affect lipid profile. Some medical conditions can raise or lower cholesterol and triglyceride levels. Cholesterol is a vital substance that the body uses to produce such things as digestion-aiding material, hormones, and cell membranes. It is both produced by the body and absorbed from some of the foods eaten. While cholesterol is necessary for various bodily functions, too much cholesterol is harmful, since excess cholesterol can be deposited in blood vessel walls. These fat deposits can lead to atherosclerosis, or hardening of the arteries, and cardiovascular disease. Induction of alloxan will increase the serum levels of total cholesterol, low density lipoprotein, triglyceride and decrease the serum level of high density lipoprotein (Bagdade *et al.*, 1991) which increases the risk for heart disease and stroke. This common condition is called diabetic dyslipidemia.

In the first lipid profile test carried out on the 9th day, there was decrease in serum triglyceride concentration in all the groups (glibenclamide, low dose and high dose groups) when compared to the control group reflecting the effect of the ethanolic leaf extract. Due to the fact that triglyceride is the most preferred source of energy when glucose is depleted, it is mobilized back to the stored depot (adipose tissue), thereby accounting for the decrease in triglyceride concentration in the blood as well as significant difference between the blood glucose level and serum triglyceride concentration. There was decrease in total cholesterol in all the groups which may also be due to the effect of ethanolic extract of *A. occidentale* (young leaves). The concentration of high density lipoprotein (HDL) increased in group 2 (glibenclamide) compared to control which may also be due to the presence of phytochemicals in the ethanolic extract and also, the improvement in the HDL may be due to improvement in the antioxidant status (Dallatu *et al.*, 2009) but it decreased in group 3 (low dose) and significantly decreased in group 4 (High dose), this could be as a result of inadequate time to respond to full treatment, this was in contrast to the previous study which said that administration of the extract significantly enhanced HDL levels (Kamtchouing, 1998; Ojewole, 2003). However, in certain cases of diabetes, treatment with insulin with normalization of plasma glucose levels did not restore the high density lipoprotein (HDL) concentrations to normal implying that, factors in addition to hyperglycemia are causing the lower HDL (Hollenbek *et al.*, 1986). The level of low density lipoprotein (LDL) increased in group 2 (glibenclamide), group 3 (low dose) and group 4 (high dose), this could also be as a result of inadequate time to respond to full treatment. This was also in contrast to the previous study carried out which said that treatment with *A. occidentale* produced significant reduction in LDL (Kamtchouing, 1998; Ojewole, 2003).

In the 2nd lipid profile test carried out, the concentration of triglyceride decreased among all the groups (glibenclamide, low dose and high dose groups). The concentration of high density lipoprotein also decreased, this could be as a result of metabolic syndrome which is a cluster of metabolic abnormalities, and is characterized by abdominal obesity, insulin resistance (IR), dyslipidemia, elevated blood pressure, one of the hallmarks of metabolic syndrome is depressed levels

of high-density lipoprotein cholesterol (Ford *et al.*, 2002). Total cholesterol increased among all the groups, the concentration of low density lipoprotein decreased in glibenclamide group and also in high dose group but the concentration of low density lipoprotein was high in low dose group compared with that of control group. This was in contrast to the previous study carried out which said that treatment with *A. occidentale* produced significant reduction in LDL and TC and produced increase in HDL (Kamtchoung, 1998).

There was decrease in weight when alloxan was administered in the low dose group and was significantly decreased in the 2nd, 4th, 6th, 14th and 16th day. There was an increase in weight in the glibenclamide group on the 2nd day which may be due to the mobilization of fats back to the adipose tissue and lipogenesis, but subsequently, the weight began to decrease and was significantly decreased on the 12th, 14th and 16th day. There was also decrease in weight in high dose group and it may be as a result of inadequate time to respond to full treatment.

Mortality rate was seen to occur in group 5 (alloxan untreated) this was as a result of high increase in blood glucose which was left untreated. The mortality rate was also seen in other groups (although very little), this may be as a result of initial low response to treatment (Bagdade *et al.*, 1991).

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