

EFFECTS OF CINNAMON AQUEOUS EXTRACT ON BLOOD GLUCOSE LEVEL, LIVER BIOMARKER ENZYMES, HEMATOLOGICAL AND LIPID PROFILE PARAMETERS IN ALLOXAN- INDUCED DIABETIC MALE ALBINO RATS.

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Abstract

Cinnamon extract are widely used in Middle East and Asian countries as herbal medication for diabetes. The study evaluates the effects of Cinnamon aqueous extract on blood glucose level, liver biomarker enzymes, hematological and lipid profile parameters in alloxan- induced diabetic male albino rats (Wistar strain). Adult male albino rats weighing between 170-220g were induced intraperitoneally with alloxan. The male albino rats were grouped into five groups of six animals per group. Group A is the normal control group, Group B served as the negative control, Group C served as positive control and was treated with glibenclamide, Group D and E were treated with 100 and 200 mg/kg body weight of cinnamon aqueous extract respectively. The extracts were given to the animals orally for 14 days. At the end of the experimental period, the albino rats from each experimental group were starved for 16 hours and sacrificed by cervical dislocation. The weight of diabetic untreated rats (Group B) were significantly ($P<0.005$) reduced when compared to other groups. The animals treated with glibenclamide, 100 and 200mg/Kg body weight of cinnamon extract showed significant decrease ($P<0.05$) of blood sugar level compared to the untreated rats (group B). This suggests that the plant extract possesses anti-diabetic and hypoglycemic effect. The extracts significantly increased RBC, HGB and HCT; and the WBC was significantly reduced in the treated groups compared to the untreated group. There were significant decrease ($P<0.05$) in plasma TC, TG, LDC-Cholesterol and an increase in HDL-Cholesterol values was observed in the treated groups compared to the untreated group. This is an indication that the extract had hypolipidemic effect and can be used in the treatment of diabetes. The extract significantly increased ($P<0.05$) plasma total protein level in the treated groups. The extract significantly reduced ($P<0.005$) liver biomarker enzymes (AST, ALT and ALP), an indication that it does not have effect on the liver.

Keywords: Alloxan-induced diabetic rats, Cinnamon aqueous extract, hematological parameters, hypoglycemic effect, lipid profiles and liver biomarker enzymes

Introduction

Diabetes mellitus (DM) is one of the major complex and chronic disorders of carbohydrate, lipid, and protein metabolism characterized by persistent elevation of blood glucose, resulting from a partial or complex cessation of insulin secretion or synthesis, or

peripheral resistance to insulin action. In diabetic patients, the body loses insulin producing capacity as a result of pancreatic β - cell apoptosis or insulin insensitivity. The cytokines, lipotoxicity and gluco-toxicity are three major stimuli for β -cell apoptosis (Hui *et al* 2004). Medicinal plants constitute an effective source of both traditional and modern medicines, herbal medicine has been shown to have genuine utility and about 80% of rural populations depend on it as primary health care. Diabetic nephropathy is one of the major causes of morbidity and premature mortality in patients with insulin-dependent DM. Medicinal plants contain potentially useful chemicals that serve as basis for the manufacturing of modern medicines (Okigbo *et al.*, 2009). Cinnamon is the bark of the *Cinnamom cassiae*, it contain cinnamom anhydride,tannin, cinnamic acid and methyl-hydroxychalcone polymer (MHCP) etc. Cinnamon has a long history as an anti-diabetic spice, but trials involving cinnamon supplementation have produced contrasting results (Kirkham *et al* 2009). Mang *et al* 2006 show that cinnamon extract seems to have a moderate effect in reducing fasting plasma glucose concentrations in diabetic patients with poor glycaemic control.

Methodology

Cinnamon extract preparation

Cinnamon aqueous extract was extracted based on method of sheng *et al* 2008. Cinnamon powder 200g was dissolved in 1000 ml double distilled water then subjected for revolving evaporator in vacuum state using vacuum pump till the volume of water reduced to about 50%. The supernatant was filtered using Whatman filter paper to obtain cinnamon extract.

Animals and biochemical Assay

Sources of animals

Male Wistar albino rats of 170 to 220g body weight (B.WT) were obtained from University of Lagos Idi-araba, Lagos- Nigeria. These animals were maintained under laboratory conditions of temperature (22 to 24°C), humidity (40 to 60%) and 12 hour light/12 hour dark regime at Lagos State polytechnic Ikorodu animal house. They were exposed to both food and water *ad libitum* for the entire duration of the study. All animals used for this study were maintained according to the rules and regulations outlined in accordance with NIH Guide for the care and use of laboratory animals; NIH Publication revised (1985) NIPRD Standard Operation Procedures (SOPs).

Administration of alloxan

Male albino rats (Wistar strain) of about fifteen weeks old with average weight of 186g were made diabetic by injecting them with alloxan monohydrate intraperitoneally with dosage of 150mg/kg body weight (Pari and Venkateswaran, 2002). Development of diabetes was confirmed after 72 hours of alloxanisation by using “Accucheck Active Glucometer” (Roche Diagnostics) and blood glucose test strips

Grouping of animals

The animals were grouped into five groups of six animals per group as shown below:

Group A – normal control (non-diabetic rats)

Group B- Negative control (diabetic without treatment)

Group C- Positive control (diabetic + glibenclamide)

Group D- Diabetic + 100mg/Kg B.WT of **Cinnamon aqueous extract**

Group E- Diabetic + 250mg/Kg B.WT of **Cinnamon aqueous extract**

Determination of hematological parameters

The total red blood cell (RBC), hemoglobin concentration (HGB), white blood cell count (WBC), platelet count and other hematological parameters were determined in the blood using BC-3200 Auto Hematology Analyzer in University of Lagos Teaching Hospitals (LUTH) in Idi-araba, Lagos, Nigeria.

Collection of blood samples for plasma preparation

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into heparinized tubes. The blood was later centrifuged for 10mins at 3000rpm using a centrifuge. The clear supernatant was used for the estimation of total protein, lipid profiles and liver function tests.

Determination of plasma lipid profiles

The plasma total cholesterol, triglyceride and HDL-Cholesterol were determined using Radox diagnostic kit [Trinder,1969 and Tietze, 1990]. Low density Lipoprotein-Cholesterol (LDL-C) was calculated using formula from [Friedwald, et al 1972].

Determination of liver function tests

Plasma enzymes like alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using Radox diagnostic kits. The total protein in the plasma was also determined using Radox kit.

Data Analysis

Data analysis was done using the GraphPad prism computer software. Students 't'-test and one-way analysis of variance (ANOVA) were used for comparison. A *P*-value < 0.05 was considered significant.

Results

Table 1 below shows that Cinnamon extract and the standard drug have hypoglycaemic effects on alloxan-induced diabetic rats.

Table 1. Effect of standard drug (glibenclamide) and Cinnamon extract (100 and 200mg/Kg B.W) on blood glucose level of albino rats induced with alloxan.

Groups	Initial glucose concentration (mg/dl)	Glucose conc. after Alloxan induction (mg/dl) Day 0	Glucose conc after 7 days of treatment (mg/dl)	Glucose conc. after 14 days of treatment (mg/dl)
Group A	98 ±10	104 ±11	100 ±11	107 ±13
Group B	98 ±7	436±21	443±33	461±43
Group C	96 ±10	461±32	212 ±40	97 ±14
Group D	101 ±12	417±31	289 ±85	137 ±12
Group E	93 ±11	438±49	274 ±82	123 ±11

Group A animals were not induced with Alloxan while Group B were induced and not treated.

The standard drug (glibenclamide), reduces the blood sugar level by 79.9% while 100 and 200 mg/Kg body weight of cinnamon extract reduce the blood sugar level by 61.1% and 67.8% respective (Table 1 above).

Determination of animal body weight

There is a progressive decrease in the body weight of diabetic untreated rats compared to other rats in other groups. As expected, alloxan-induced diabetic rats showed all the characteristic of diabetes such as polyuria, polyphagia, polydipsia and loss of body weight.

This is evident from figure I, below. Group B (diabetes untreated animals) albino rats have significant (P<0.05) weight lost compared to other groups.

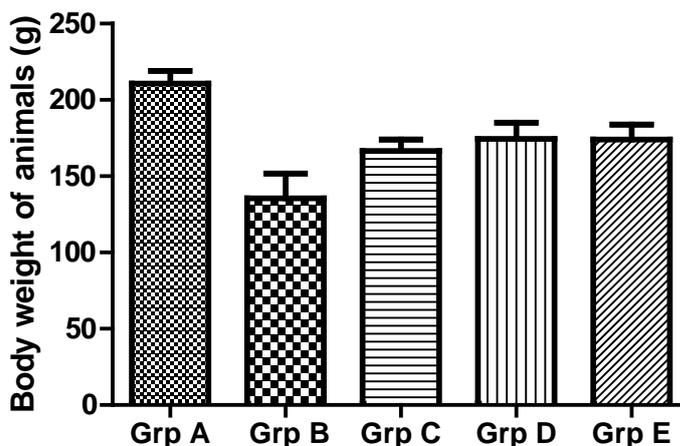


Figure 1: Mean body weight (g) of normal, Diabetic untreated, Diabetic rats treated with glibenclamide, 100 and 200mg/kg body weight of Cinnamon extract.

Table 2. The effect of Cinnamon extract and glibenclamide on hematological parameters of male albino rats induced with alloxan..

Hematological parameters	GROUP A	GROUP B	GROUP C	GROUP D	GROUP E
WBC ($\times 10^9/L$)	9.3 ± 1.1*	15.2±2.1	11.1±2.3*	10.2±1.4*	9.8 ±1.2*
HGB g/dl	13.1 ± 1.3*	8.9 ± 2.6	12.2 ± 1.2*	13.8 ± 1.6*	14.4 ± 1.9*
RBC ($\times 10^{12}/L$)	7.8 ± 1.6*	5.3 ± 1.8	7.0 ± 1.2*	6.9± 0.8*	7.4 ± 1.8*
HCT %	44.3 ± 2.1*	29.2 ± 7.2	46.1 ± 1.1*	49.3 ± 1.5*	47.2 ± 1.8*
MCV fl	62.0 ± 1.0	59.1 ± 2.4	59.3 ± 0.5	62.3 ± 1.2	58.8 ± 1.2
MCH pg	19.1 ± 1.3	18.2 ± 0.8	18.5 ± 1.1	19.2 ± 0.9	19.1 ± 1.1
MCHC g/dl	31.2 ± 0.8	31.7 ± 1.0	31.6 ± 1.2	29.4 ± 1.1	31.1 ± 0.7
RDW-CV %	16.8 ± 0.6	16.1 ± 0.7	16.9 ± 0.8	16.4± 1.1	16.7 ± 0.9
RDW-SD fl	35.5 ± 1.1	32.1 ± 1.5	33.6 ± 0.8	38.4 ± 1.4	36.7 ± 0.7
MPV fl	6.7 ± 0.4	7.9 ± 0.8	7.1 ± 0.5	7.0± 0.4	6.5 ± 0.4
PDW	16.4 ± 0.7	16.3 ± 0.4	16.0 ± 0.4	16.1 ± 0.3	15.8 ± 0.6
PCT %	0.411 ± 0.017	0.420 ± 0.023	0.345 ± 0.011	0.361 ± 0.154	0.386 ± 0.199

The values are the Means ± SD for six rats in each group. White blood count (WBC), Hemoglobin (HGB), Red blood count (RBC), Hematocrit (HCT), Mean cell volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red Blood Cell Distribution Width Coefficient of Variation (RDW-CV), Red Blood Cell Distribution Width Standard Deviation (RDW-SD), Mean platelet volume (MPV), platelet Distribution Width (PDW) and Plateletcrit (PCT).

The different hematological parameters of the entire experimental groups (group A to E) are shown in Table 2 above.

Table 3. The effect Cinnamon extract on lipid profiles in alloxan-induced diabetic male rats.

Parameters	Group A	Group B	Group C	Group D	Group E
Total Cholesterol (mg/dl)	71.3 ± 4.5*	98.4 ± 6.5	80.1 ± 6.7*	75.3 ± 5.3*	73.5 ± 4.9*
Triglyceride (mg/dl)	82.4 ± 3.2*	143.2 ± 4.30	104.2 ± 6.8*	99.4 ± 5.9*	93.5 ± 4.8*
Low-density Lipoprotein (mg/dl)	10.1 ± 0.5*	44.6 ± 1.60	22.6 ± 2.4*	16.4 ± 2.8*	13.6 ± 3.1*
High-density Lipoprotein (mg/dl)	46.2 ± 4.2*	25.3 ± 5.70	37.2 ± 3.4*	39.6 ± 4.8*	40.2 ± 2.9*

* indicate Significant difference (P < 0.05) when comparing normal and treated group with negative control group.

Cinnamon extract significantly reduces ($P<0.05$) the plasma level of TC, TG and LDL-Chol in the treated animals compared to the untreated animals. The plasma HDL-Chol of the treated animals is enhanced by the administration of the extracts (Table 3).

The effect of Cinnamon extract on plasma liver marker enzymes and total protein in healthy and alloxan-induced diabetic male rats.

Figure 2 below shows that cinnamon extract significantly reduces plasma Aspartate aminotransferase (AST) in alloxan-induced diabetic rats.

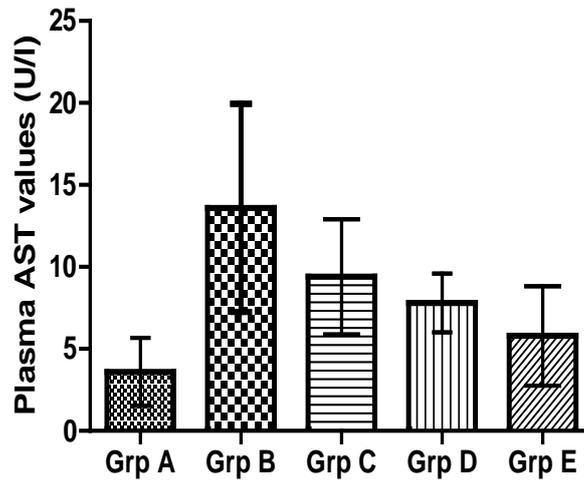


Figure 2. Plasma Aspartate aminotransferase (AST) values of normal, Diabetic untreated, Diabetic rats treated with glibenclamide, 100 and 200mg/kg body weight of Cinnamon extract.

Figure 3 below shows that cinnamon extract significantly reduces plasma Alanine aminotransferase (ALT) in alloxan-induced diabetic rats.

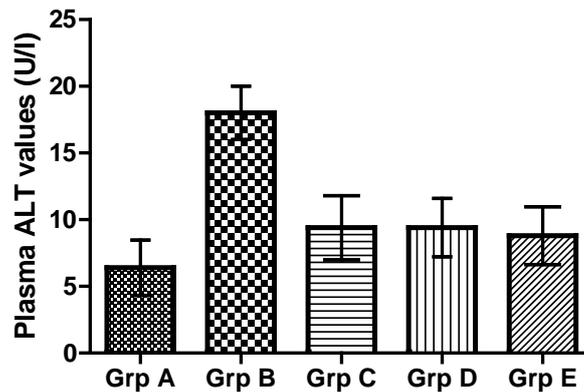


Figure 3. Plasma Alanine aminotransferase (ALT) values of normal, Diabetic untreated, Diabetic rats treated with glibenclamide, 100 and 200mg/kg body weight of Cinnamon extract.

Figure 4 below shows that cinnamon extract significantly reduces plasma Alkaline Phosphatase (ALP) in alloxan-induced diabetic rats.

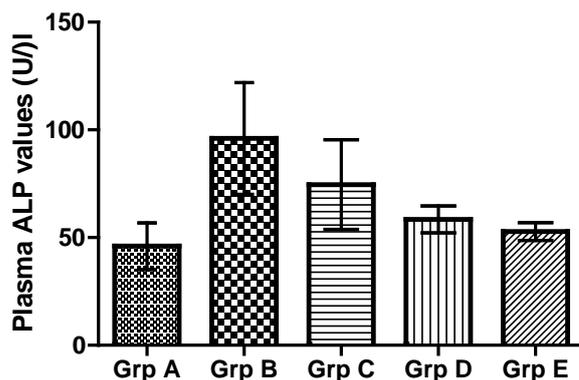


Figure 4. Plasma Alkaline Phosphatase (ALP) values of normal, Diabetic untreated, Diabetic rats treated with glibenclamide, 100 and 200mg/kg body weight of Cinnamon extract.

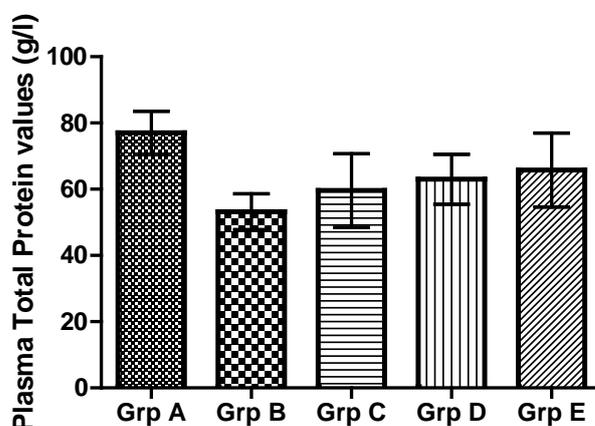


Figure 5. Plasma Total Protein values of normal, Diabetic untreated, Diabetic rats treated with glibenclamide, 100 and 200mg/kg body weight of Cinnamon extract.

Figure 5 above shows that cinnamon extract significantly increases plasma total protein in alloxan-induced diabetic rats.

Discussion

Several hypoglycemic herbs have been used as non-prescription treatment for diabetes. Few herbal medicines have been shown to have hypoglycaemic effect, however their test result is subjected to several factors. First each herb contains thousands of components, only a few of which may be therapeutically effective. Secondly extraction of active component is not easy (Karashima, 1988 and Angelova et al, 2008). Ebong *et al.*, 2008 showed clearly that medicinal plants formed the basis of health care throughout the world and have considerable importance. Some of these herbal preparations have been found to exert biological actions against diabetes mellitus and its complications (Ojiako and Nwanjo, 2006). Alloxan induce diabetes by damaging the insulin secreting cells of the pancreas leading to hyperglycemia (Szuldelski et al 2001).

Table 1 shows significant decrease in blood sugar level after periods of 7 and 14 days of treatment. The results showed that cinnamon extract exhibited a significant reduction ($P < 0.005$) in blood glucose level of the diabetic male albino rats. The hypoglycaemic effect of cinnamon oil (CO) in a type 2 diabetic animal model was studied by Ping *et al* 2010 and according to their study CO was administrated at doses of 25, 50 and 100 mg/Kg for 35 days. They found that fasting blood glucose concentration was significantly decreased ($p < 0.05$) with the 100 mg/Kg group ($p < 0.01$) compared to diabetic control group. They observed that CO had a regulative role in blood glucose level and lipids, and improved the function of pancreatic islets. A number of other plants have been reported to have antihyperglycemic and insulin stimulatory effects. (Venkateswaran S, 2002 and Latha M, Pari L: 2003)

Graph 1 above shows that there was a significant reduction ($P < 0.005$) in the body weight of group B untreated diabetic rats compared to other rats in other groups. This could be due to the defect in glucose metabolism and excessive breakdown of tissue protein which is a characteristic condition of diabetics (Swanston-Flat. *et al* 1990).

There was a significant reduction ($P < 0.05$) in WBC levels of diabetic rats treated with cinnamon aqueous extract compared to the untreated albino rats. This shows the abilities of the above treatment groups to curtail hematological abuses in the defense system of the diabetic rats. The study shows that there is a significant increase ($p < 0.005$) in the level of RBC, HGB and HCT of group C and the treated groups compared to the untreated group (Table 2). These may be as a result of anemia or the onset of glycosylation process in the untreated diabetic rats (group B). The extract may not have adverse effects on the bone marrow, kidney and hemoglobin metabolism, since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and hemoglobin metabolism (Young and Maciejewski 1997). The non- significant change ($P < 0.05$) in the MCV and MCH values indicate absence of macrocytic anemia since increased in MCV an MCH values are known to be indicative of macrocytic anaemia. The extract also caused non- significant change in the MCHC value which suggests absence of hereditary spherocytosis since MCHC values are known to be elevated in hereditary spherocytosis. Other hematological parameters (RDW-CV, RDW-SD, PLT, MPV, PWD and PCT) showed no significant differences in the entire different group.

Table 3 shows that cinnamon extract lowered plasma TC, TG, LDL-Chol levels and increase HDL-Chol concentrations in the treated rats, and this could account for its use in traditional medicine for the treatment of diabetes and hypertension. The results of this study clearly indicate that the administration of cinnamon extract produces hypoglycemic and hypolipidaemic effect and may prevent cardiovascular diseases. Studies have shown that increased in the risk factor of cardiovascular disease correlate with increase in plasma TC, TG, LDL-Chol, atherogenic index level and a decrease in HDL-Chol concentrations. The results of an experimental study show that cinnamaldehyde possesses hypoglycaemic and hypolipidemic effects in STZ-induced diabetic rats (Blevins *et al* 2007). This is in agreement with our studies. These results suggest that cinnamon aqueous extract has a regulatory role in reducing blood glucose level and lipids parameters. This may be due to blood glucose suppressing effect by improving insulin sensitivity or slowing absorption of carbohydrates in the small intestine.

The extract caused significant decrease ($P < 0.05$) in the activity of AST, ALT and ALP values as shown in Figure 2-4. These indicate that the extract may reduce hepatic damage. In medicine, the presence of elevated values of ALT and AST is indicative of liver damage (Giboney, 2005). ALP levels in plasma rise as a result of large bile duct obstruction, intrahepatic cholestasis or infiltrative diseases of the liver. Increased level of ALP has been attributed to the damaged structural integrity of hepatic cells because the enzyme alkaline phosphatase is located in the cytoplasm and is released into the circulation after cellular

damage. The plasma total protein increases in the healthy group and treated groups compared to the untreated diabetic group (Figure 5).

Conclusion

The results show that aqueous extract of cinnamon possesses hypoglycaemic effect, hypolipidemic properties and the extract is not hematotoxic and may reduce liver damage induced by alloxan.

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