# Evaluation of the Effects of *Colatropis gigantea* Leaf Extracts on Blood pH, Blood Glucose and Total Protein concentrations in Diabetic Rabbits.

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**ABSTRACT:** Screening for the most effective organic extract revealed that acetone fraction significantly decreased the blood glucose level ($p<0.05$) when compared to other fractions and therefore was used for further study in phase 11. The result showed reduction of blood pH with significant value ($p<0.05$) of diabetic untreated when compared to group treated with acetone fraction of *C. gigantea* leaf extract. There was no significant difference ($p>0.05$) in blood pH among the extract-treated groups and glibenclamide-treated group. Protein concentration was observed to increase significantly ($p<0.05$) in diabetic rabbits treated with *C. gigantea leaf* extract when compared to diabetic untreated group. A significant decrease ($p<0.05$) in value of protein concentration of the group treated with acetone fraction of *C. gigantea* leaf extract was observed when compared with group treated to reference drug (glibenclamide).

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**Keywords:** *Colatropis gigantea*; Blood pH; Blood Glucose; Total Protein; Diabetic Rabbits.

## INTRODUCTION

The quality of life and the life span of the patients with the disease depend on its complications. Hence, there is an increased interest in dealing with this disorder. Convincing evidences of the role of free radicals and oxidative stress in the pathogenesis and complications of diabetes mellitus have been established over times. It was shown that the patients were put under increasing oxidative stress in conjunction with different biochemical defects – the inactivation of nitric oxide, which is key to maintaining vascular tones. Significant changes in lipid metabolism and structure also occur in diabetes. In these cases the structural changes are clearly oxidative in nature and are associated with development of vascular disease in diabetes (Baynes *et al.*, 1999).

Diabetes mellitus is the most important disease involving the endocrine pancreas. Its major manifestations include disordered metabolism and inappropriate hyperglycaemia. Currently there are over 150 million diabetics worldwide and this number is likely to increase to 300 million or more by the year 2025 due to increase in sedentary lifestyle, consumption of energy rich diet, and obesity (Yajnik, 2001). While management of diabetes mellitus includes diet, exercise, oral hypoglycaemic agents, and insulin, these treatments do not effectively prevent the complications of diabetes like nephropathy, neuropathy, cataract, and hypertension (Palumbo, 2001).

Random, or "casual," plasma glucose measurement is inexpensive, easily accomplished, and free of risk and discomfort, except for risks from phlebotomy. The test can be performed at the same clinic visit as the patient evaluation. Sources of error relate to technical problems, such as delayed centrifugation (McIntyre *et al.*, 1997), and medications that alter serum glucose levels (National Diabetes Data Group, 1979). In the absence of metabolic decompensation, a patient with a random plasma glucose level of 11.1 mmol/L or higher (≥200 mg/dL) and classic symptoms of diabetes should have a second confirmatory test to diagnose diabetes. Measurement of fasting plasma glucose level requires the patient to fast overnight for at least 8 hours, which can be inconvenient and requires a follow-up clinic visit. Otherwise, it is inexpensive and risk-free. The accuracy of test results may be compromised by patient nonadherence to fasting or laboratory error; moreover, use of certain medications can affect test results (National Diabetes Data Group, 1979). Fasting plasma glucose levels vary considerably within individuals over the long term (Muggeo *et al.*, 1997) but are relatively stable over the short term. The results of fasting plasma glucose measurement are substantially more reproducible than those of the OGTT. Studies that have directly compared the two tests have found intra-individual coefficients of variation of 6.4% to 11.4% for measurement of fasting plasma glucose and 14.3% to 16.7% for measurement of 2-hour plasma

glucose (Feskens *et al*., 1991). The implications for the overall prevalence of diabetes inherent in lowering the fasting plasma glucose threshold from 7.8 mmol/L (140 mg/dL) to 7.0 mmol/L (126 mg/dL) are somewhat unclear. Given universal screening (for example, in one epidemiologic study), use of one fasting plasma glucose value with the lower threshold would lead to fewer diagnoses of diabetes than would use of the OGTT. However, because the OGTT is infrequently used in clinical practice (Orchard, 1994), the lowering of the fasting plasma glucose threshold will probably increase the prevalence of diabetes diagnosed in clinical practice.

The study is primarily aimed at evaluating the effect of the extract of *Colatropis gigantea* on blood pH, blood glucose and total protein concentrations in both normal and diabetic rabbits.

## MATERIALS AND METHODS

### Chemicals

The chemicals used for this study are of analytical grades and include ethanol, methanol, n-hexane, butanol, chloroform, acetone, ethylacetate, ethylene diamine tetracetate (EDTA), hydrochloric acid, sulphuric acid, sodium hydroxide, trichloroacetic acid, (TCA) adrenaline, 2-thiobarbituric acid (TBA), alloxan monohydrate (sigma-Aldrich, USA) and 1 chloro-2,4-dinitrothiobenzene, glutathione peroxidase kit (Randox Laboratories Limited, United Kingdom), Protein kit (Randox Company, USA). Glucose test (Life Scan Inc, California, USA)

### Drug

The known hypoglycaemic drug used was glibenclamide 10mg/kg body weight.

### Plant Material

The leaves of the plant *Colatropis gigantea* were obtained from Ogadimma Research Farm in Effiom, Ebonyi state. The plant was identified at Department of Botany, University of Nigeria, Nsukka.

### Animals

The animal care and handling was done according to the guidelines set by the World Health Organization, Geneva, Switzerland. Healthy, adult male rabbits with an average body weight of 700±50 g obtained from the animal house of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria were used for the study. he mice used for $LD_{50}$ determination were 4-6 weeks old with average weight of about 25.50 ± 0.50g. The animals were housed at room temperature (25±3$^{o}$C) with a 12 h reverse light cycle and had free access to water and food (standard laboratory diet).

### Experimental design

The study was made up of two phases.

### Phase I

Phase I was the screening phase at which the most potent fractionated extract amongst the fractions was noted and used in the second phase.

Diabetes was induced by slow intraperitoneal injection of 1% solution of alloxan (200mg/kg body weight) in normal saline and administered within few minutes of preparation. Diabetic state was confirmed after three days using glucometer. The animals were administered with the extract twice daily intraperitoneally at a dose of 300mg/kg body weight for five days. Screening was done by estimating the fraction that reduced the elevated blood glucose level close to normal using glucometer.

A total of eighteen (18) rabbits were used and was divided into nine groups:

- Group 1: Normal rabbits fed on normal rabbit chow and water ad libitum.
- Group 2: Diabetic rabbits not treated and given water *ad libitum.*
- Group 3: Diabetic rabbits treated with glibenclamide (standard) and water *ad libitum*
- Group 4:Diabetic rabbits treated with n-hexane fraction and water *ad libitum.*
- Group 5: Diabetic rabbits treated with chloroform fraction and water *ad libitum.*
- Group 6: Diabetic rabbits treated with ethylacetate fraction and water *ad libitum.*
- Group 7: Diabetic rabbits treated with butanol fraction and water *ad libitum.*
- Group 8: Diabetic rabbits treated with acetone fraction and water *ad libitum.*
- Group 9: Diabetic rabbits treated with methanol fraction and water *ad libitum.*

### Phase II

This Phase of the study was made up of four (4) groups of three (3) rabbits each. Diabetic induction was done as described in the phase I. Treatment was administered twice daily intraperitoneally at a dosage of 300mg/kg body weight for five (5) days. Blood samples were collected through ear vein for biochemical analysis. Liver and pancreatic tissues were dissected out, washed in normal saline and kept in formal calcium removed for histopathological study.

A total of twelve rabbits were used and divided into groups.

Group 1:Normal rabbit fed on normal rabbit chow and water *ad libitum.*

Group 2:Diabetic rabbits not treated and water *ad libitum.*

Group 3:Diabetic rabbits treated with Glibenclamide (Standard) and water *ad libitum.*

Group 4: Diabetic rabbits treated with most active fraction (acetone fraction) and water *ad libitum*

**Plant treatment**

Fresh leaves of *C. gigantea* were dried under room temperature, crushed and soaked in 200ml of analytical grade of methanol for 48 hours then filtered and was evaporated with rotary evaporator at room temperature to obtain the crude extract.

**Extraction procedure**

The crude extract was subjected to fractionation using different organic solvents. It was first extracted using n-hexane. The n-hexane soluble fraction was obtained and concentrated using rotary evaporator at an optimum temperature of 25°C. The resulting residue (residue – A) was dried and then fractionated using chloroform. The soluble chloroform fraction was concentrated using rotary evaporator and obtained chloroform extract while the resulting residue (residue B) was subsequently fractionated using ethyl acetate. The ethyl acetate soluble fraction was concentrated using rotary evaporator and an insoluble residue (residue C). The insoluble residue obtained was re-suspended in acetone, filtered and the filtrate concentrated. The residue obtained (residue D) was further fractionated using methanol. The methanol soluble fraction was concentrated and the methanol concentrate was obtained while the insoluble portion obtained was dried; this was soluble in water.

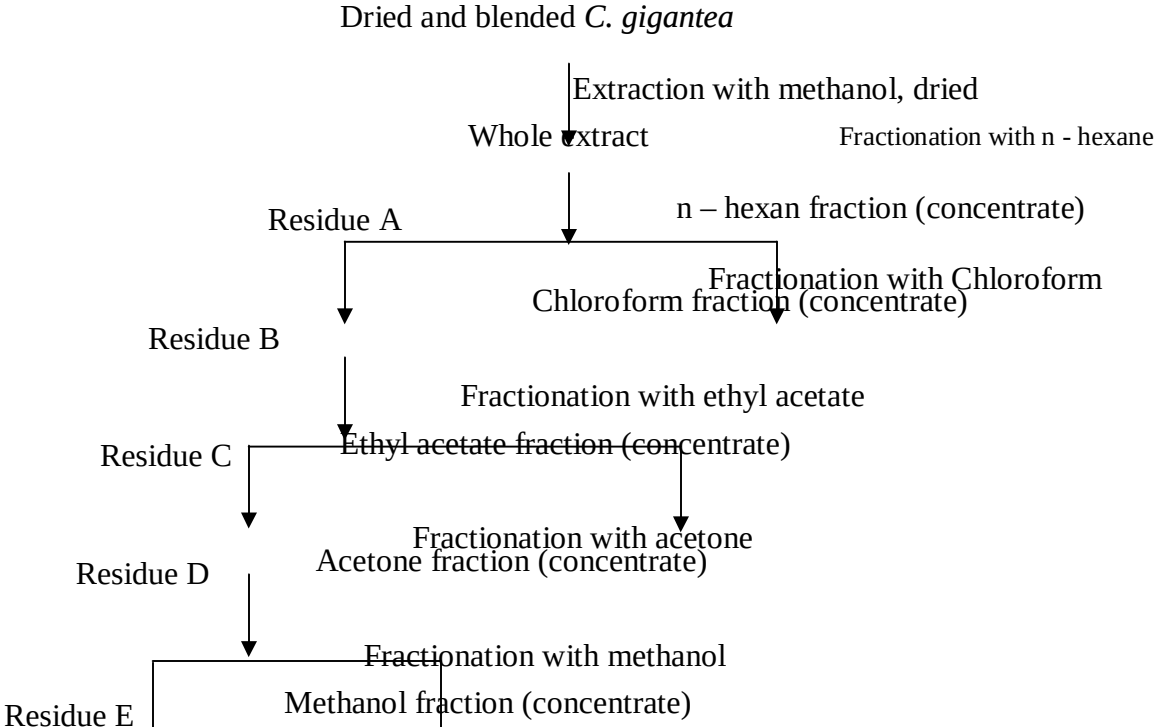


Fig. 1: Fractionation of *C. gigantea* leaves

**Determination of Yield of Extract**

$$\% \text{ Yield} = \frac{\textit{Weight (g) of Extract Evaporated}}{\textit{Weight (g) Ground Sample Pulp}} \times 100$$

**Anti-diabetic Evaluation**

**Induction of Diabetes**

**Phase I**

Twenty four rabbits with sugar concentrations of 60-90mg/dl after 12 hours of fasting were injected intraperitoneally (i.p.) with 200mg/kg body weight of freshly prepared alloxan monohydrate (Sigma, USA) in normal saline. The animals were fed with Bendel Feed and Flour Mill Limited Pelletized Guinea Growers mash.

After three days, blood sugar concentrations were determined using glucometer. Blood sugar concentrations found to be 150mg/dl and above were used for determination of hypoglycemic effects of *C. gigantea* leaves extract. The diabetic rabbits were divided into eight groups of two animals each. The extract dissolved in the normal saline and was injected intraperitoneally.

Group 1 represented the control group, while group 2 represented diabetic not treated as described in the experimental design. Group 3 received glibenclamide as standard hypoglycaemic agent. Groups 4 – 9 received different fraction of the extract at the dose of 300mg/kg body weight twice daily for five days.

After five days treatment, Groups 4 – 9 were screened down to one group using blood sugar level as a marker that determined the most active fraction.

**Phase II**

Twelve rabbits having sugar concentration of 60 – 90 mg/dl after 12 hours of fasting were injected intraperitoneally (i.p.) with 200 mg/kg body weight of freshly prepared alloxan monohydrate (Sigma, USA) in normal saline. After 3 days, blood sugar was determined using glucometer. Blood sugar levels were found to be 150 mg/dl and above. The diabetic rabbits were divided into 4 groups of 3 rabbits each. Group 1 represented normal control; group 2 represented diabetic untreated; group 3 represented diabetic treated with glibenclamide (reference drug) while group 4 received the acetone fraction (most active fraction). After 5 days treatment, blood samples were collected through the ear veins of the animals for oxidative parameter assay.

**Methods**

**Determination** of **Serum Glucose Concentration**

The one touch glucose monitoring meter (life scan inc. Johnson - Johnson Company, Mulpiter California, USA) was used as described in the operation manual. A drop of whole blood (0.04ml) was placed on a strip connected to the glucometer. The glucometer automatically separates serum from blood cells and determined the blood glucose.

This method was based on the reaction of glucose and oxygen in the presence of glucose oxidase to yield gluconic acid and hydrogen peroxide.

Hydrogen peroxidase subsequently metabolizes $H_2O_2$ producing a blue coloured product, the colour intensity of which was proportional to the glucose concentration in the sample, which was read from the one – touch glucometer.

**Determination of Total Protein Concentration**

The determination of total protein concentration was done according to the method of Slater (1986.).

***Principle:***

This method is based on the principle that cupric ions, in an alkaline medium, interact with peptide bonds of proteins resulting in the formation of a coloured complex.

**Determination of Blood pH**

Test strips (Medi-Test combi 9) for rapid determination of blood pH was used.

***Principle:***

The determination is based on the pseudoperoxidative activity of hemoglobin and myoglobin, which catalyze the oxidation of an indicator by an organic hydroperoxide producing a green colour.

***Procedure:***

Dip the reagent strip for approximately 1 second in the fresh blood. Test paper contains indicators which clearly change colour between pH 5 and pH 9 (from orange to green to turquoise).

**Statistical Analysis**

The results were expressed as mean ± SD and tests of statistical significance were carried out using student t-test and both one-way and two-way analysis of variance (ANOVA). The means were separated using Duncan Multiple Test. The statistical package used was Statistical Package for Social Sciences (SPSS); version 18.

## RESULTS

**Effect of the Fractionated Extracts of *C. gigantea* on Glucose Level in Diabetic Rabbits**

The data in Table 1 below depict the respective effects of 300 mg/kg body weight of different fractionated extract on mean fasting blood glucose level of diabetic rabbits. The effect on the blood glucose levels was the basis of determining the most potent fraction of *C. gigantea* leaves.

Acetone fraction significantly ($P<0.05$) lowered blood glucose levels of diabetic rabbits when compared to other solvent fractions.

**Effect of Acetone Fraction of *C. gigantea* Leaf Extract on Total Protein Concentration**

Total protein concentration was observed to increase significantly ($p<0.05$) in diabetic rabbits

treated with *C. gigantea leaf* extract when compared to diabetic untreated group (Fig. 2). A significant decrease ($p<0.05$) in value of protein concentration of the group treated with acetone fraction of *C. gigantea* leaf extract was observed when compared with group treated to reference drug (glibenclamide).

**Effect of Acetone Fraction of *C. gigantea* Leaf Extract on Blood pH**

The result showed reduction of blood pH with significant value ($p<0.05$) of diabetic untreated when compared to group treated with acetone fraction of *C. gigantea* leaf extract. However, there was no observable difference in the blood pH value of both groups treated with glibenclamide and group treated with acetone fraction of *C. gigantea* leaf extract.

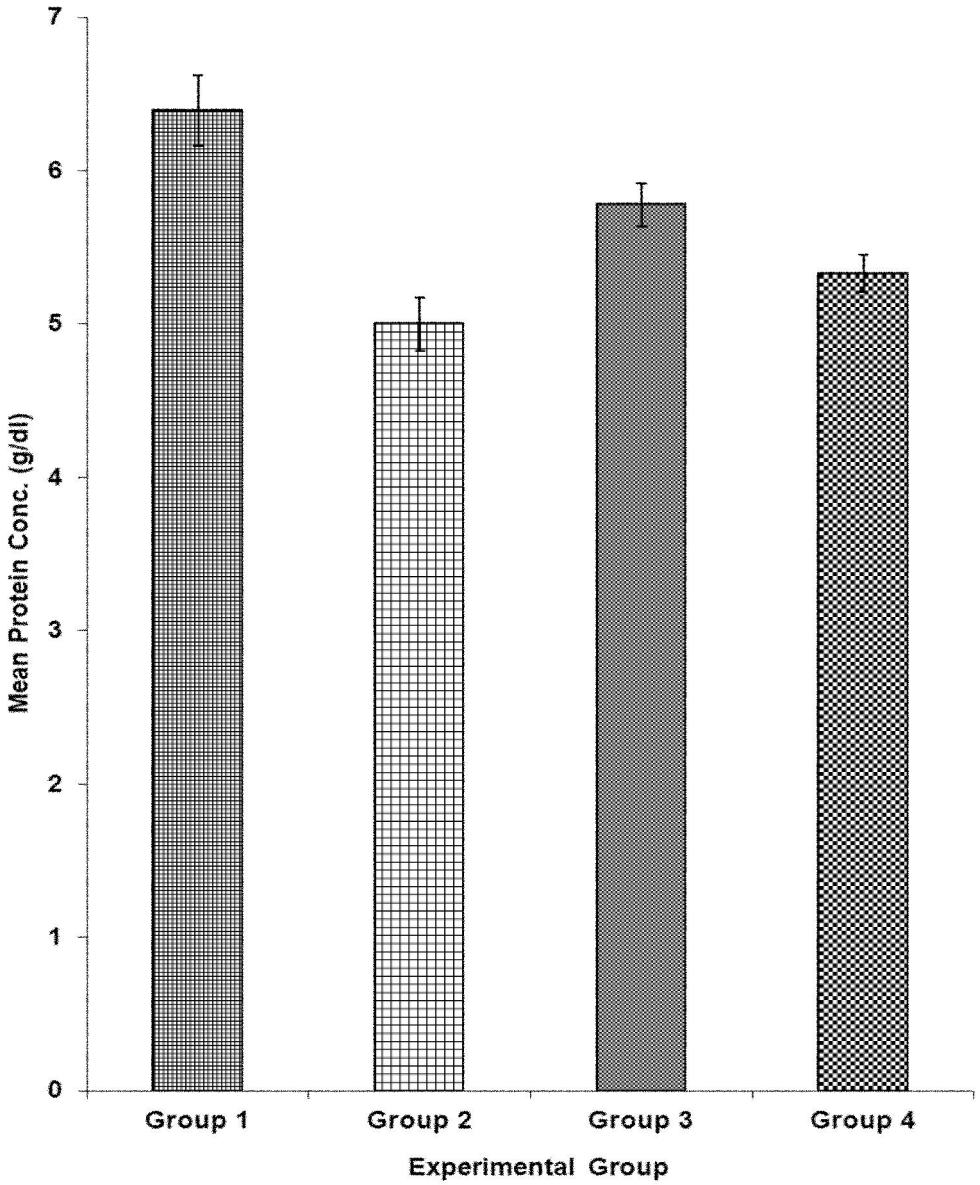


Fig. 2: Effect of acetone extract of *C. gigantea* leaf on the total protein concentration in rabbits

| | |
|---|---|
| Group 1 = Control; | Group 3 = Diabetic treated with Glibenclamide |
| Group 2: Diabetic Untreated; | Group 4 = Diabetic treated with Acetone fraction |

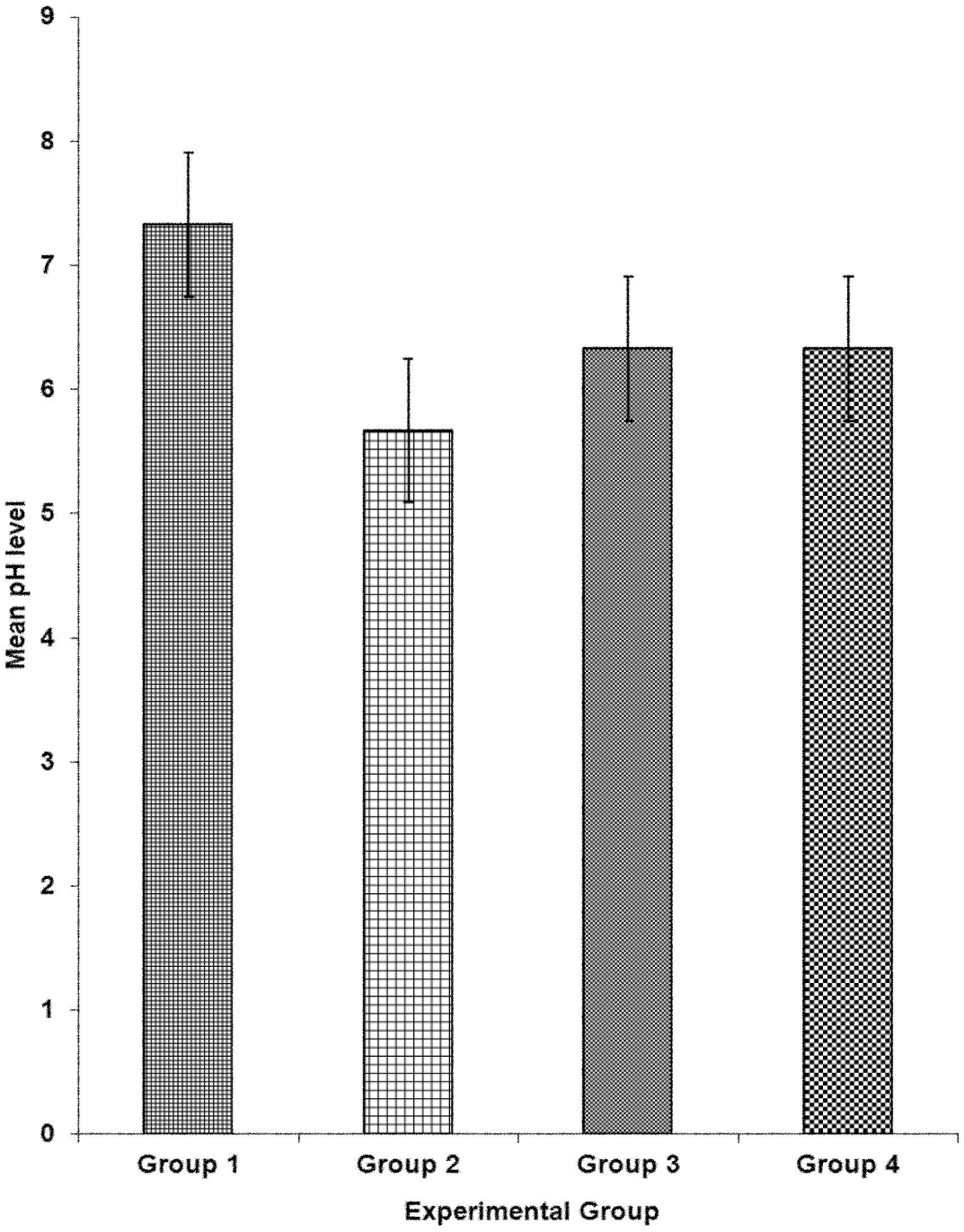


Fig. 3: Effect of acetone extract of *C. gigantea* leaf on the blood pH concentration in rabbits

| | |
|---|---|
| Group 1 = Control; | Group 3 = Diabetic treated with Glibenclamide |
| Group 2: Diabetic Untreated; | Group 4 = Diabetic treated with Acetone fraction |

**Phase I**

Table 1: Effect of solvent fractions of *C. gigantea* leaf on glucose level

| Treatment Group | Mean FB sugar level (mg/dl) | Mean sugar level (mg/dl) after induction | Mean sugar level (mg/dl) after treatment | % Maximum reduction |
|---|---|---|---|---|
| 1 (Control) | 95.00±4.24 | – | – | – |
| 2 (Diabetic not treated) | 84.00±2.83 | 249.00±1.41 | 236.00±2.83 | 5.20 |
| 3 (Diabetic treated with standard drug) | 96.50±0.71 | 153.00±1.41 | 112.00±4.24 | 26.80 |
| 4 (Diabetic treated with n-hexane fractions | 87.00±1.41 | 152.00±5.66 | 150.00±2.83 | 1.30 |
| 5 (Diabetic treated with chloroform fraction) | 88.50±0.71 | 204.50±2.12 | 203.50±0.71 | 0.75 |
| 6 (Diabetic treated with ethylacetate fraction | 74.50±4.95 | 174.00±5.66 | 166.00±1.41 | 4.60 |
| 7 (Diabetic treated with butanol fraction) | 98.50±0.71 | 307.50±0.71 | 290.50±20.51 | 5.53 |
| 8 (Diabetic treated with acetone fraction) | 95.50±3.54 | 153.00±7.07 | 127.50±0.71 | 16.99 |
| 9 (Diabetic treated with methanol) | 74.00±5.66 | 165.00±7.07 | 281.00±7.07 | 14.70 |

**Phase II**

Table 2: Effect of acetone fraction of *C. gigantea* leaf on glucose level

| Treatment Group | Mean FB sugar level (mg/dl) | Mean sugar level (mg/dl) after induction | Mean sugar level (mg/dl) after treatment | % Maximum reduction |
|---|---|---|---|---|
| 1 (Control) | 90.00±4.24 | – | – | – |
| 2 (Diabetic untreated) | 98.50±3.54 | 239.00±15.56 | 236.00±7.07 | 25.10 |
| 3 (Diabetic treated with standard drug) | 98.00±7.07 | 184.50±2.12 | 115.00±4.24 | 37.20 |
| 4 (Diabetic treated with acetone) | 98.50±6.36 | 308.00±31.11 | 205.50±27.58 | 33.30 |

## DISCUSSION

Diabetes mellitus is probably the fastest growing metabolic disease in the world and as the knowledge of the multifactorial/heterogenous nature of the disease increases so does the need for more challenging and appropriate therapies. Traditional plant remedies have been used for centuries in the treatment of diabetes (Akhtar and Ali, 1984), but only a few have been scientifically evaluated.

In phase I it appeared that acetone fraction at the dosage of 300mg/kg body weight reduced blood sugar more than other solvent fractions. However, glibenclamide at 10g/kg (a reference drug) could reduce blood sugar slightly above that of acetone extract which informs the use of this plant as an anti-diabetic agent. Although the mechanism of action of the extracts has not been investigated, some scientists have suggested that the hypoglycaemic principle of some extracts work by binding to the ATP-Inhibited $K^+$ channel in the β-cells membrane. This is meant to inhibit the channel activity which depolarizes the β-cell membrane and increase $Ca^{2+}$ influx to stimulate more insulin release (Ganong, 2001).

The results indicated that the administration of acetone fraction of *C. gigantea* leaf extract improved the alloxan effect, but did not completely normalized the diabetogenic action induced by allozan when compared with control group. Adephate (1999b) showed that the increase in the tissue concentration of glucagons might play a significant role in the development of hyperglycaemic. It is believed that long-term administration of the plant extract could provide an opportunity for regeneration of more insulin-producing beta cells with subsequent decrease of glucagons immuno-expression and normalization of the blood glucose concentration.

Alloxan induces hyperglycaemia by selective cytotoxic effect on pancreatic β-cells (Szkudelski, 2001) causing permanent destruction of β-cells. The dosage of 200mg/kg of alloxan used in this study caused moderate diabetes (Grover *et al.*, 2000). It has been reported that glibenclamide was not very effective after the occurrence of complete destruction of β-cells; hence more effective in the moderately diabetic rabbits than in severe diabetes (Sharma *et al.*, 1997). The acute hypoglycaemic effect of glibenclamide has been shown to be by the stimulation of production of the residual β-cells of the pancrease in addition to enhancement of glucose utilization (Moller, 2001). This suggests that the extracts may have a similar mechanism of action with glibenclamide and may in addition possess an isulinomimetic effect on peripheral tissue (i.e.

extrapancreatic mechanism) either by promoting glucose uptake and metabolism or inhibiting hepatic gluconeogenesis (Djomeni, 2006). This postulation correlates with that of Farjou *et al*. (1987) on the work with Artemisia.

The antihyperglycaemic activities of the acetone fraction of *C. gigantea* leaf extracts were quite considerable and found to be close to that of glibenclamide; and this inferred that this extract may be more efficacious than glibenclamide as more than one component may be responsible for the activity. The evidence reported that the attributed antihyperglycaemic effects of most plants are due to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to facilitate the metabolite in insulin dependent process (Elder, 2004; Jia *et al*., 2003).

Statistical significance ($p<0.05$) was observed in the serum protein concentration in the diabetic rabbits treated with *C. gigantea* leaf extracts compared with diabetic control. Normal rabbits showed higher protein concentration compared with diabetic treated with *C. gigantea* leaf extract and diabetic treated with glibenclamide.

The result of the blood pH indicated increase in the blood pH (i.e. towards normal) of the diabetic rabbits treated with acetone fraction of *C. gigantea* leaf extract and group treated with glibenclamide when compared with normal. There was no significant difference ($p>0.05$) between diabetic rabbits treated with acetone fraction of *C. gigantea* and group treated with glibenclamide. It can be inferred that increased blood levels of acetoacetate and D-β-hydroxybutyrate observed in diabetic condition lowered the blood pH, thereby causing the condition known as acidosis in the untreated group.

In conclusion, the results of this study have shown that leaves of *C. gigantea* possess antihyperglycaemic effect. Intraperitoneal administration of *C. gigantea* leaf extract revealed their action on glucose metabolism. The protective effect of *C. gigantea* leaf extract may be connected with the normalization of hyperglycaemia and the inhibition of autoxidation.

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