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HEPATOPROTECTIVE EFFECTS OF METHANOL LEAF EXTRACT OF *Napoleonae imperialis* IN STREPTOZOTOCIN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

This study aimed to evaluate the hepatoprotective effects of methanol leaf extract of *Napoleonae imperialis* in streptozotocin induced diabetic albino rats. Forty two (42) male albino rats of mean weight 140 g were used for the study. The animals were grouped into seven (7) groups of six (6) rats each. Group 1 is the normal control that received only feed and water. Group 2 is the diabetic untreated group. Group 3 is drug control group. Groups 4, 5 and 6 were the test groups that were orally given 250, 500 and 1000 mg/kg body weight of the methanol leaf extract, group 7 is the group that received the extract only (500 mg/kg b.wt). Treatment lasted for 28 days, after which the animals were sacrificed under mild anesthesia. Blood samples were collected for biochemical analysis. The result from the study showed that there was statistically significant ($p< 0.05$) decrease in the fasting blood glucose concentration of the rats in the test groups, when compared with the diabetic untreated group. The result also showed that there was statistically significant ($p> 0.05$) decrease in the concentration of ALT, AST, ALP, total bilirubin and albumin in the treated groups, when compared with the diabetic untreated group. The results of this study indicate that methanol leaf extract of *Napoleonae imperialis* possesses antidiabetic and hepatoprotective effects in albino rats, and thus could be utilized pharmacologically in the management and treatment of diabetes and organ toxicity.

KEYWORDS: *Napoleonae imperialis*; streptozotocin; glibenclamide; antidiabetic; hepatoprotective effects.

1. INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by the elevation of blood sugar level (hyperglycemia), hyperlipidemia or dyslipidemia, and glycosuria, generally due to insulin resistance or insulin deficiency. There are several etiological factors involved in the development of type 2 diabetes (noninsulin-dependent diabetes mellitus), namely, genetic factor, age, diet, obesity, and lifestyle. Since the last couple of decades, it was observed that type 2 diabetes has become a global health problem. As estimated by the World Health Organization, more than 176 million people are suffering from this disease globally.[1] In the Western countries, diabetes mostly develops with advancement in age, but in Asian countries, it affects mostly young to middle-aged population. Various associated macrovascular (cerebrovascular and cardiovascular diseases) and microvascular complications (neuropathy, retinopathy, and nephropathy) develop during diabetes. It has been suggested that enhanced generation of free radicals, oxidative stress, and deficiency of endogenous antioxidants are the cardinal issues in the development of diabetic complications.[2]

Studies have shown that diabetes elicits redox imbalance of endogenous antioxidant system of our body by overproduction of free radicals (reactive oxygen and nitrogenous species) that are responsible for various cellular and subcellular detrimental changes, thus aggravating the condition. Recent studies suggest that implication of antioxidants in diet or as medicine is an alternative tool for the management of diabetic complications. The plant kingdom offers an untapped source of antioxidant agents which require thorough exploitation.[3,4]

Herbal drugs currently are the most acceptable alternative medicine for the majority of the world population both in the developing and in the developed countries.[5] There is an increase in the use of herbal remedies for the treatment of various diseases especially among the rural populace in the developing countries, which are mostly attributed to their potency and their availability as a cheap source of medical treatments.[6] There exists cumulating evidence that medicinal plants serve as valuable starting materials for drug development in both developed and developing countries most especially in Africa where it acts as the first line of treatment of various diseases for more than 80% of her population and various parts of *Napoleonae imperialis* have also been used.[7]

Napoleonaea imperialis is a little, perennial humid West African plant in the family Lecythidaceae, native to Africa.[8]

The plant grows to about 6 m in height, with a thick, low-branching crown, and spreads from Benin, Nigeria, Gabon and the Democratic Republic of the Congo southwards to Angola. The attractive buds have two interior rows of petals and differ in colour, generally creamy yellow beside the edge, and the middle stretching from red to apricot to purple.[9] Extracts gotten from the leaves exhibit bactericidal property and comprises of glycosides, tannins, phenols, alkaloids, terpenoids, saponin, flavonoids, reducing sugar, carbohydrate and steroids.[10] It is used in the treatment of wound and hypertension among the indigenous locals in Eastern Nigeria where the plant is commonly found and that it does not cause any significant harms to those individuals that consume it. This study was designed to evaluate the hepatoprotective effects of methanol leaf extract of *Napoleonae imperialis* in streptozotocin induced diabetic albino rats.

2. MATERIALS AND METHODS

Drugs and chemicals

Streptozotocin (STZ) from Sigma (Sigma, USA), glibenclamide tablets (Daonil) from Aventis Pharma Limited, Goa, India, and analytical grade solvents were obtained from Rankem (Rankem, Pvt. Ltd, New Delhi). All other chemicals and reagents used were of analytical grade.

Plant material

Fresh leaves of the plant *Napoleonae imperialis* was obtained from a local farm in Umuariaga village, Umudike, Abia State, Nigeria, and was identified by Dr. Garuba Omosun, a Taxonomist of the Plant Science and Biotechnology Department, Michael Okpara Uiversity of Agriculture, Umudike. The fresh leaves of the plant collected was washed and dried under shade at room temperature and were milled to fine powder using an electric blender (QLink, Model QBL, Taiwan) and stored in air tight containers.

Extraction

The powdered leaves of *Napoleonae imperialis* 1000 g (1 kg) was soaked in methanol for 48 hours, after which the extract was filtered using a Whatman no. 1 filter paper and then the filtrate was air dried and stored in the refrigerator for further use as methanol leaf extract of

Napoleonae imperialis. During the experiment the crude extract was diluted with distilled water just before administration to the animals.

Animals

Healthy looking male albino rats of mean weight of 140 g were used for the study. All animals were kept in metabolic cages in the animal house under normal room conditions and acclimatized for two (2) weeks. Commercial pellet diet (Vital growers mash by Grand Cereals and Oil Mills, Nigeria) and water were given to the animals *ad libitum*.

Induction of experimental animals

All the rats were fasted overnight before the administration of streptozotocin. Diabetes was induced in rats by intra peritoneal injection of streptozotocin dissolved in 0.1 M sodium citrate buffer pH 4.5 at the dose of 65 mg/kg body weight. After the injection they had free access to food and water. The animals were allowed to drink 5% glucose solution overnight to overcome hypoglycaemic shock. The development of diabetes was confirmed after 48 hours of Streptozotocin injection. The animals with fasting blood glucose level more than 200 mg/dL were considered as diabetic rats and used for the experimentation.

Experimental design

Forty two (42) male albino rats of mean weight 140 g were used for this study. The animals for the study were group into seven (7) groups of six (6) rats each. Group 1 was the normal control that received feed and water only, group 2 was the negative control (diabetic untreated group) and group 3 was the positive control (drug control that received 5 mg/kg body weight of the standard drug glibenclamide). Test groups (4), (5) and (6) were orally given 250, 500 and 1000 mg/kg body weight of methanol leaf extract of *Napoleonae imperialis* and group 7 was the group that received the methanol leaf extract of *Napoleonae imperialis* only (500 mg/kg b.wt). All the rats used in this study were initially subjected to diabetes by single intraperitoneal induction of 65 mg/kg body weight of streptozotocin except the normal control (group 1) and the group that received the extract only (group 7). Treatment last for 28 days and after which the animals were sacrificed under mild anesthesia (10% formasaline). Blood samples were collected in the plain bottle for the analyses on the hepatoprotective effects of methanol leaf extract of *Napoleonae imperialis* in streptozotocin induced diabetic albino rats.

Determination of blood glucose concentrations

The blood glucose concentrations was determined using ACCU-CHEK Active Glucometer by Roche Diagnostic by glucose oxidase-peroxidase method.[11]

Alanine aminotransferase (ALT) activity assay: Determination of serum ALT activity was done using the method of.[12]

Aspartate aminotransferase (AST) activity assay: Determination of serum AST activity was done using the method of[12]

Alkaline phosphatase (ALP) activity assay: Determination of serum ALP activity was done using the method of.[12]

Determination of albumin: Albumin was determined according to the method of.[13]

Determination of serum total bilirubin (T. bil) concentration: The total serum bilirubin concentration was determined using the method of.[14]

STATISTICAL ANALYSIS

The results obtained was analyzed statistically using one-way analysis of variance (ANOVA) to get the grouped mean which was used to determine the significant difference between the group means at 95% level of confidence, using the statistical products and service solutions (IBM SPSS Statistics 22.0), and $p \leq 0.05$ was considered significant.

3. RESULTS

Result of methanol leaf extract of *Napoleonae imperialis* on blood glucose level in streptozotocin induced diabetic albino rats

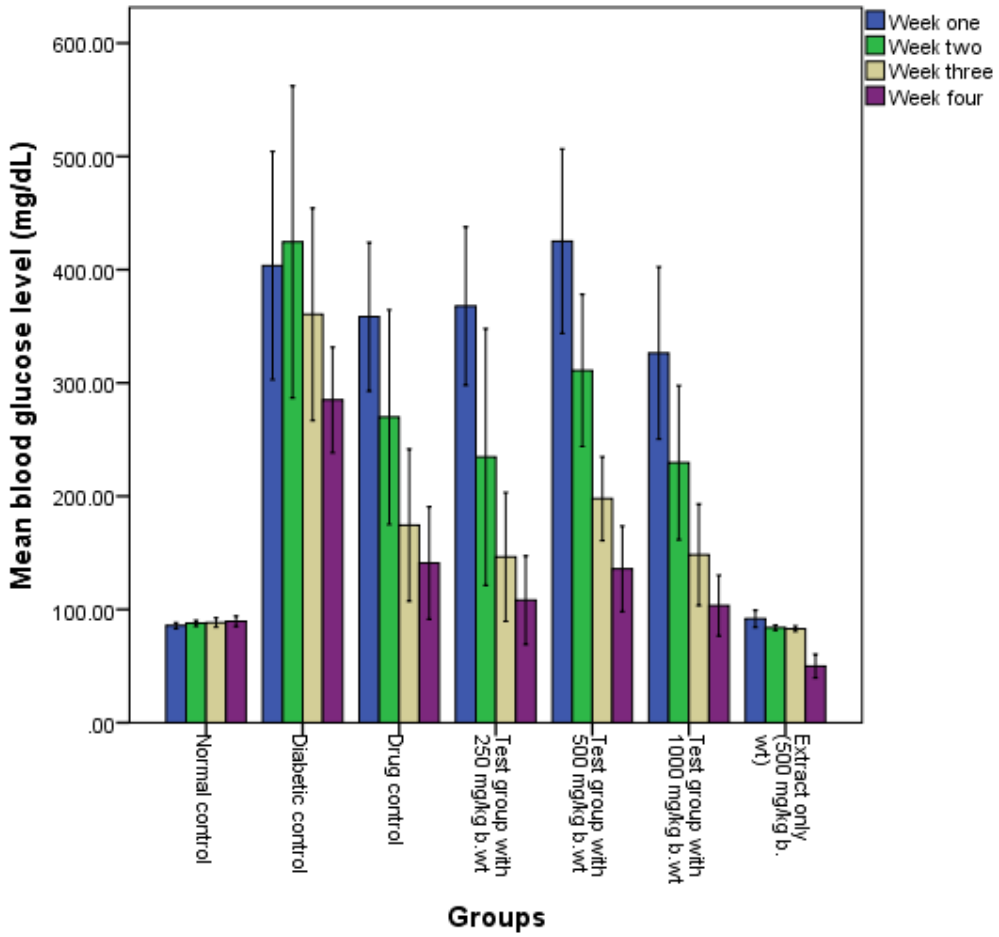

Fig. 1: Blood glucose concentration of the control and test groups.

There was statistically significant ($p<0.05$) decrease in the fasting blood sugar level from week one to week four in the treated groups that orally received 250, 500 and 1000 mg/kg body weight of the extract, drug control (5 mg/kg body weight of glibenclamide) and the group that received the extract only (500 mg/kg body weight) when compared to the diabetic untreated group, indicating that the methanol leaf extract and the standard drug were able to lower the sugar level.

Result of methanol leaf extract of *Napoleonae imperialis* on ALP activity in Streptozotocin induced diabetic albino rats

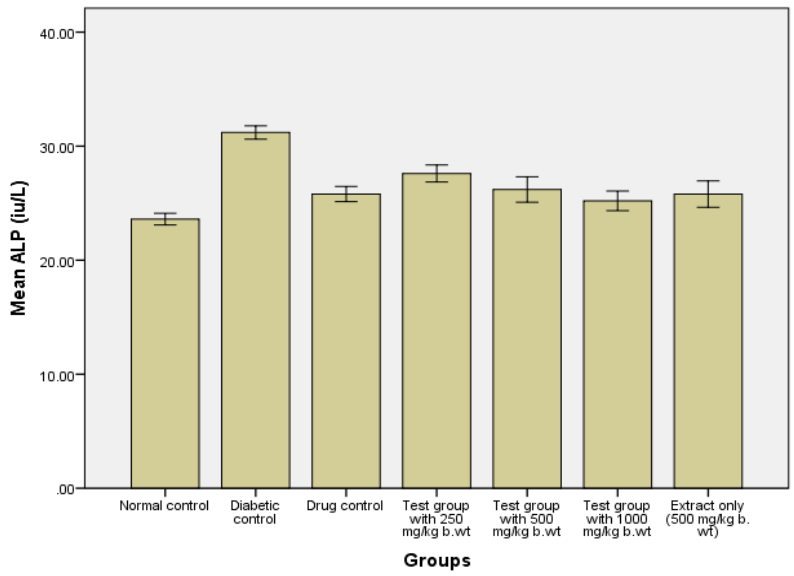

Fig. 2: Alkaline phosphatase concentration of the control and test groups.

There was statistically significant ($p < 0.05$) decrease in the test groups that orally received 250, 500, and 1000 mg/kg body weight of the extract, the normal control, the drug control that received 5 mg/kg body weight of glibenclamide, and the group that received the extract only (500 mg/kg body weight) when compared with diabetic untreated control group.

Result of methanol leaf extract of *Napoleonae imperialis* on ALT activity in Streptozotocin induced diabetic albino rats

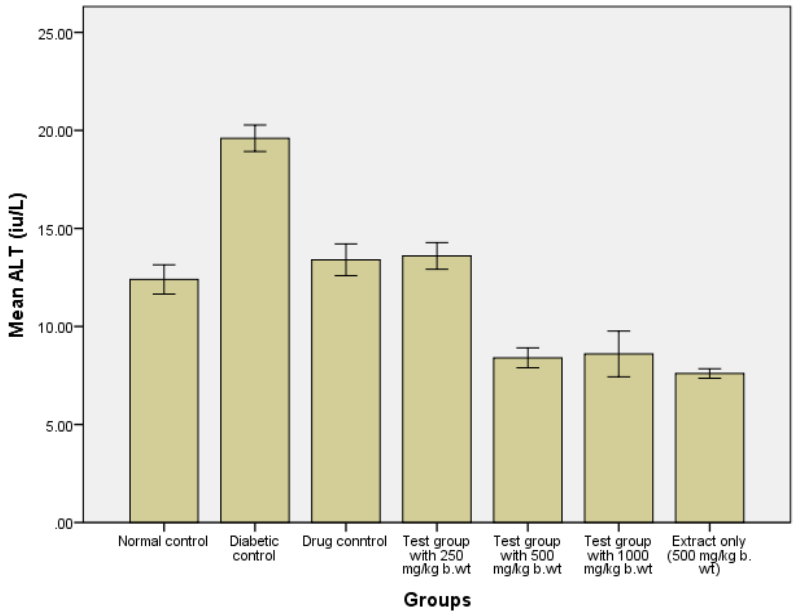

Fig. 3: Alanine aminotransferase concentration of the control and test groups.

There was statistically significant ($p < 0.05$) decrease in the test groups that orally received 250, 500, and 1000 mg/kg body weight of the extract, the normal control, the drug control that received 5 mg/kg body weight of glibenclamide, and the group that received the extract only (500 mg/kg body weight) when compared with diabetic untreated control group.

Result of methanol leaf extract of *Napoleonae imperialis* on AST activity in Streptozotocin induced diabetic albino rats

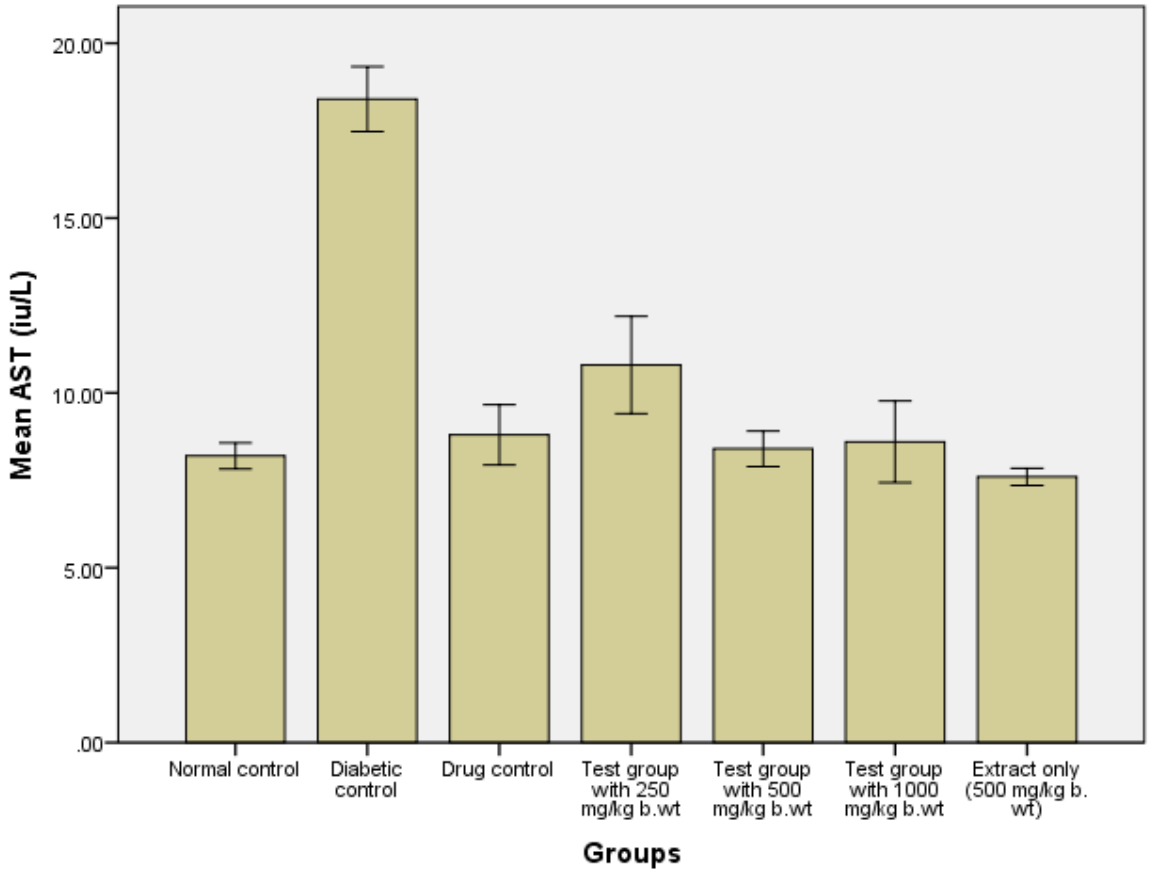

Fig. 4: Aspartate transaminase concentration of the control and test groups.

There was statistically significant ($p < 0.05$) decrease in the test groups that orally received 250, 500, and 1000 mg/kg body weight of the extract, the normal control, the drug control that received 5 mg/kg body weight of glibenclamide, and the group that received the extract only (500 mg/kg body weight) when compared with diabetic untreated control group.

Result of methanol leaf extract of *Napoleonae imperialis* on Albumin in Streptozotocin induced diabetic albino rats

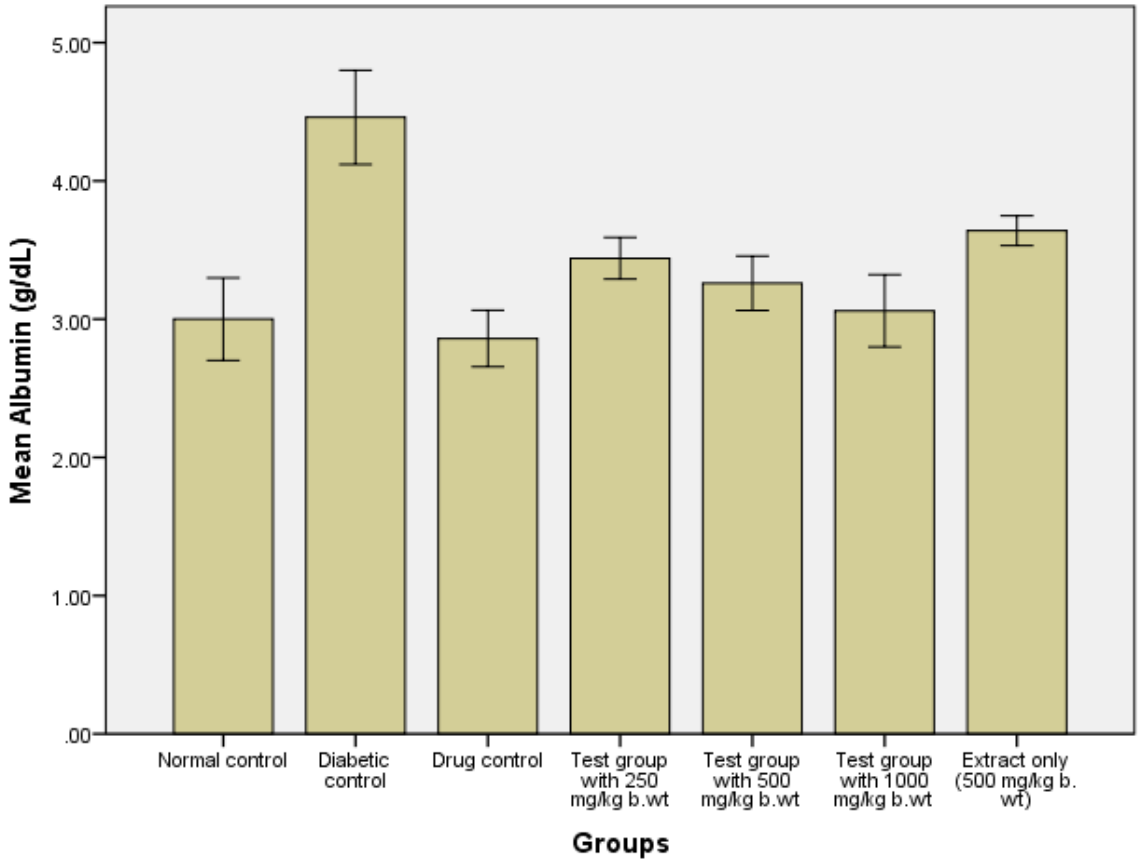

Fig. 5: Albumin concentration of the control and test groups.

There was statistically significant ($p < 0.05$) decrease in the test groups that orally received 250, 500, and 1000 mg/kg body weight of the extract, the normal control, the drug control that received 5 mg/kg body weight of glibenclamide, and the group that received the extract only (500 mg/kg body weight) when compared with diabetic untreated control group.

Result of methanol leaf extract of *Napoleonae imperialis* on Total bilirubin in Streptozotocin induced diabetic albino rats

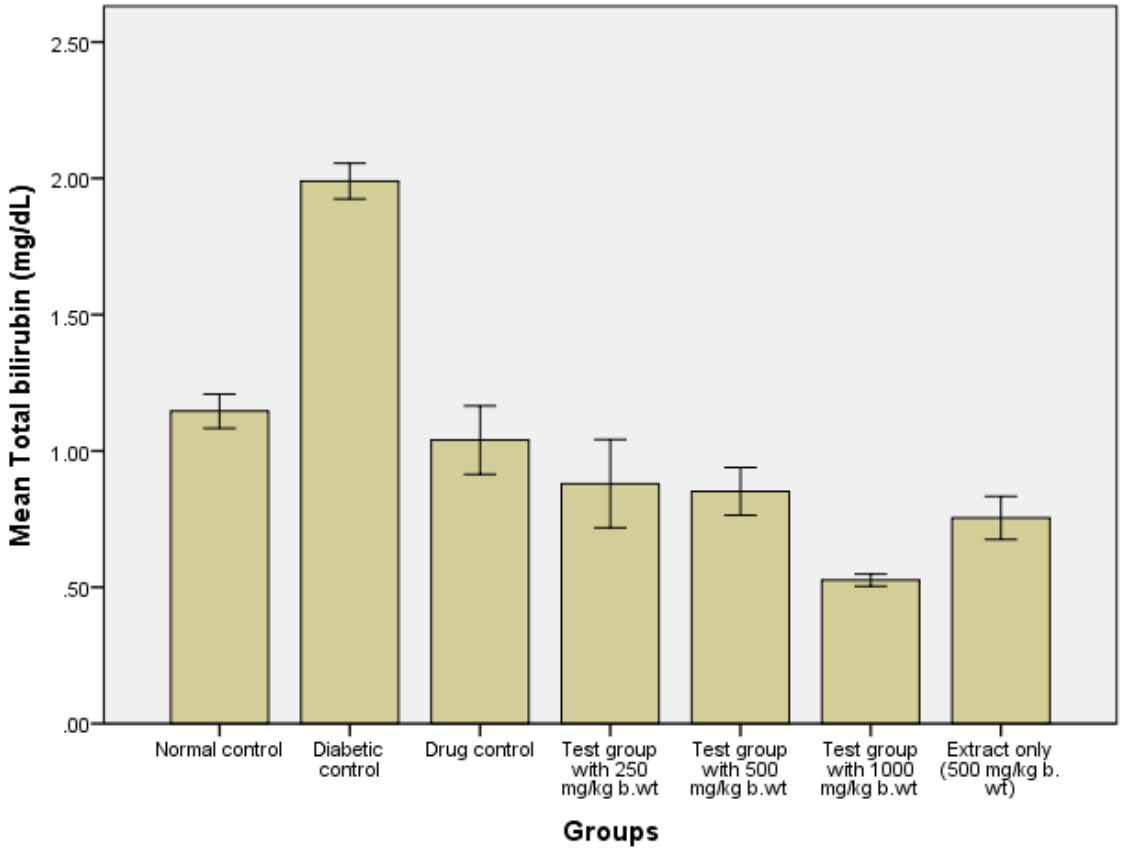

Fig. 6 Total bilirubin concentration of the control and test groups.

There was statistically significant ($p < 0.05$) decrease in the test groups that orally received 250, 500, and 1000 mg/kg body weight of the extract, the normal control, the drug control that received 5 mg/kg body weight of glibenclamide, and the group that received the extract only (500 mg/kg body weight) when compared with diabetic untreated control group.

4. DISCUSSION

In the present study, there was statistically significant increase in the concentration of fasting blood glucose in STZ induced diabetic rats as compared to normal rats, which could be as a result of destruction of beta cells of the pancreas. Administration of the methanol leaf extract of *Napoleonae imperialis* at different concentrations showed statistically significant ($p < 0.05$) reduction in blood glucose when compared with the diabetic untreated control at the expiration of the 28 days study. This study showed the ability of the extract to reduce blood glucose level which might be due to the presence of the bioactive compound such as

kaempferol which has been reported to stimulate insulin secretion and quercetin which increases hepatic glucokinase in an insulin like manner as earlier reported by.[15] The accumulating evidence suggests that modulation of insulin secretion and or insulin action could be involved in the antidiabetic effect of the leaf extract. This evidence was confirmed by[10] who revealed that *Napoleonae imperialis* leaves contains phytochemicals like flavonoids, steroids, terpenoids, phenols, tannins, glycosides, alkaloids, saponins which may stimulate insulin secretion or which protect the intact functional β-cells from further deterioration or due to regeneration of STZ damaged β-cells. For example,[16] revealed that administration of flavonoids to STZ-induced diabetic rats resulted to an elevation in the action of insulin and glucokinase in the plasma samples associated with a drop in the glucose-6-phosphatase action. Study of[17] earlier suggested that *Napoleonae imperialis* leaves may have the potential to become the lead compound in the improvement of new types of antidiabetic pharmaceuticals that are able to reduce blood glucose levels without increasing adiposity.

Liver is the vital organ of metabolism, detoxification, storage and excretion of xenobiotic and their metabolites. Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin and bilirubin are considered as part of liver toxicity markers.[18] In streptozotocin-induced diabetic animals, change in the serum enzymes is directly related to change in the metabolic functions of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin and bilirubin.[19] It has been reported that the increased aminotransferase activities under insulin deficiency were responsible for the increased gluconeogenesis and ketogenesis during diabetes.[20] Aspartate aminotransferase is an enzyme found mainly in the cell of the liver, heart[21], skeletal muscles, kidney, and pancreas and to a lesser amount in red blood cells. Its serum concentration is proportional to the amount of cellular leakage or damage and it is released into the serum in larger quantities when any one of these tissues is damaged and its increase is usually associated with heart attack or liver disease.[22] While on the other hand, alanine aminotransferase is an enzyme found mainly in the liver and elevated levels in serum usually indicates liver damage.[23] The mechanism by which the alkaline phosphatase, serum aspartate and alanine aminotransferases are raised in diabetic untreated group may involve increased liberation of these enzymes from tissues (mainly liver), owing to oxidative stress or the formation of advanced glycosylation end product.[24] The increase in the activities of these enzymes in serum of diabetic control might be induced due to liver dysfunction.

In this study, there were statistically significant ($p<0.05$) increase observed in these liver marker enzymes (AST, ALT and ALP, albumin and bilirubin) activities in the diabetic control which was streptozotocin induced but not treated suggesting that the streptozotocin might have compromised the liver integrity and function possibly through the mechanism of free radical generation by the streptozotocin. This might have damaged liver membrane and resulted to the increased permeability of the membrane leading to the leakage of the liver enzymes to the extrahepatic tissues. The significant ($p<0.05$) decrease observed in these liver marker enzymes of the methanol leaf extract of *Napoleonae imperialis* treated groups relative to the diabetic untreated group could be attributed to its antioxidant activity against free radicals generated by streptozotocin which could have caused liver damage and thus, minimized the damage caused by streptozotocin to liver architecture and function.[25] This is in line with the previous report that medicinal plants can exhibit hepatoprotective effects through additive and synergistic actions of antioxidant activities of their phytochemicals constituents like phenol and flavonoids.[10] The methanol leaf extract of *Napoleonae imperialis* could have restored the membrane permeability thereby preventing leakage of the liver enzymes to the extrahepatic tissue and indicated that the methanol leaf extract of *Napoleonae imperialis* leaves could be said to have exhibited hepatocurative activity.

The concentrations of bilirubin and albumin may indicate state of the liver and type of damage. Bilirubin is formed by the breakdown of hemoglobin in the liver, spleen and bone marrow.[26] An increase in tissue or serum albumin concentrations results in jaundice. Jaundice occurs in toxic or infectious diseases of the liver.[27] An increase in bilirubin level could be attributed to three major causes such as hemolysis, biliary obstruction and liver cell necrosis.[28] The significant increase in the total bilirubin, albumin levels in the diabetic control rats and reduction following oral administration of methanol leaf extract of *Napoleonae imperialis* are indicative of amelioration of the adverse effects caused by diabetes.[29]

CONCLUSION

The findings of this study indicate that methanol leaf extract of *Napoleonae imperialis* possesses antidiabetic and hepatoprotective effects and has potential as hypoglycemic agent and exhibited significant improvement in blood glucose level capable of maintaining liver integrity and functions through stabilization of membrane as observed in the decreased amount of liver marker enzymes.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85- 23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropiate ethics committe.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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