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Assessment of the anti-diabetic potential of ethanol leaf extract of *Crateva adansonii* in streptozotocin induced diabetic rats

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Abstract

Diabetes mellitus is a group of metabolic disorders causing disturbance in the metabolism of lipids, carbohydrate and protein. Synthetic drugs used in the treatment of diabetes are known to have adverse effects. Thus the aim of this research work was to evaluate the anti-diabetic of ethanol leaf extract of *Crateva adansonii* in diabetic rats. Freshly harvested leaf of *Crateva adansonii was dried and subsequently processed into* extract. Twenty five (25) adult male wistar rats were divided into five groups of five rats each. Group I was the Normal control (NC), Group II was diabetic untreated rats, Group III and IV were diabetic rats treated with 200 and 400 mg/kg of *C. adansonii* respectively. Intraperitoneal injection of streptozotocin caused a significant ($P<0.05$) increase in blood sugar level. However, oral administration of 200 and 400 mg/kg of extract significantly ($P<0.05$) reduced blood sugar level compared to the level reported for the diabetic untreated Group II rats though higher than that reported for the normal control. In conclusion, it can be deduced from this study that ethanol leaf extract of *C. adansonii* wield anti-diabetic potential which could be enhanced with higher doses of the aforementioned extract.

Keywords: Diabetes; *Crateva adansonii*; Streptozotocin; Sugar; Blood

1 Introduction

Diabetes mellitus is a metabolic disorder that adversely affects lipids, carbohydrate and proteins metabolism. Its pathophysiology is traced to defective insulin production and or target-tissue resistance and increased hepatic glucose output [1]. DM has been implicated in retinopathy, neuropathy, nephropathy and cardiovascular diseases and accounts for mortality and morbidity in both developed and developing countries. It is expected to be the 7th leading cause of death in 2030 [2]. Generation of reactive oxygen species by the mononuclear cells is cardinal to the pathophysiology of diabetes mellitus which translates to damage to cellular materials such as DNA, protein, lipids, carbohydrates and consequently, impaired metabolic activities [3]. An estimated 20% of plants have been explored by mankind to improve health and wellbeing [4]. Research efforts have revealed that plants are appreciably endowed with antioxidants which are undoubtedly critical in mitigating the damaging effects of free radicals [5]. This is evident by the fact that 80% of the populations of developing countries rely on plant based therapies to treat human diseases [6].

Crateva adansonii DC, a member of the *Capparidaceae* family is a plant that wields impressive therapeutic significance and has been found useful in the treatment of bacterial infection, sore, abscesses, high blood pressure and rheumatism etc. [6]. Owing to the fact that many studies have revealed the hypoglycemic activity of extracts derived from different parts of other members of the *Capparidaceae* family, it is also imperative to explore the leaf of *C. adansonii* in the treatment of experimentally induced diabetes in an effort to further unveil the therapeutic significance of the aforementioned plant.

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2 Material and methods

2.1 Collection and Identification of Plant Material

Fresh leaf of *Crateva adansonii* was obtained from a local market in Nasarawa Local Government area of Nasarawa State Nigeria. The leaf was subsequently identified at the herbarium unit of the Department of Biological Sciences, Ahmadu Bello University Zaria.

2.2 Extraction of the Plant Material

Fresh leaves of *C. adansonii* were spread on a clean flat surface and allowed to dry at room temperature for 14 days and afterwards ground into fine powder. Exactly 450 g of the powdered leaf sample soaked in 2500 mL of aqueous: ethanol (30:70, v/v) was intermittently stirred and shaken and re-macerated thrice daily for 3 days. This followed by filtration with the aid of a Whatman no. 1 filter paper, the combined filtrate was evaporated and concentrated using a rotary evaporator and kept in a ventilated oven at 40 ^{0}C until dried. The dried extract was placed in a sealed container and stored in a refrigerator at 4 °C.

2.3 Animals

Adult male wistar rats weighing between 150-250g were obtained from the animal house of the Department of Pharmaceutical Science, ABU Zaria. The rats were housed adequately ventilated cages 12/12 light and dark cycles. They were maintained on grower's mash (Vital feeds Nigeria Ltd) and provided with water *ad libitum*. Acclimatization of animals lasted for two weeks prior to the commencement of the experiment.

2.4 Acute Oral Toxicity Study

Acute oral toxicity test was performed on ethanol leaf extract of *C. adansonii* in accordance with the method described by the Organization for Economic Cooperation and Development (OECD) 425 guideline 26 using five healthy female mice (20-30 g) which had been acclimatized for 14 days. At first, a single mouse was fasted for 4 h and afterwards administered with 2000 mg/kg of the extract orally and observed periodically for 24 h for any acute sign of toxicity. However, the remaining four animals were administered with same dose and cage side observation was made for gross behavioral changes for 14 days. Then, the dose was raised to 5000 mg/kg.

Twenty five (25) adult male wistar rats weighing between 150-250g divided into five groups of five rats each.

Group I: (Normal control) was fed rat chow and water.

Group II: Diabetic-induced rats without treatment (negative control).

Group III: Diabetic-induced rats administered with 200 mg/kg bw of the extract.

Group IV: Diabetic-induced rats administered with 400 mg/kg bw of the extract

Group V: Diabetic-induced rats administered with a standard drug (metformin).

After 28 days of administration, animals were humanely sacrificed and blood sample was collected.

2.5 Induction of Diabetes

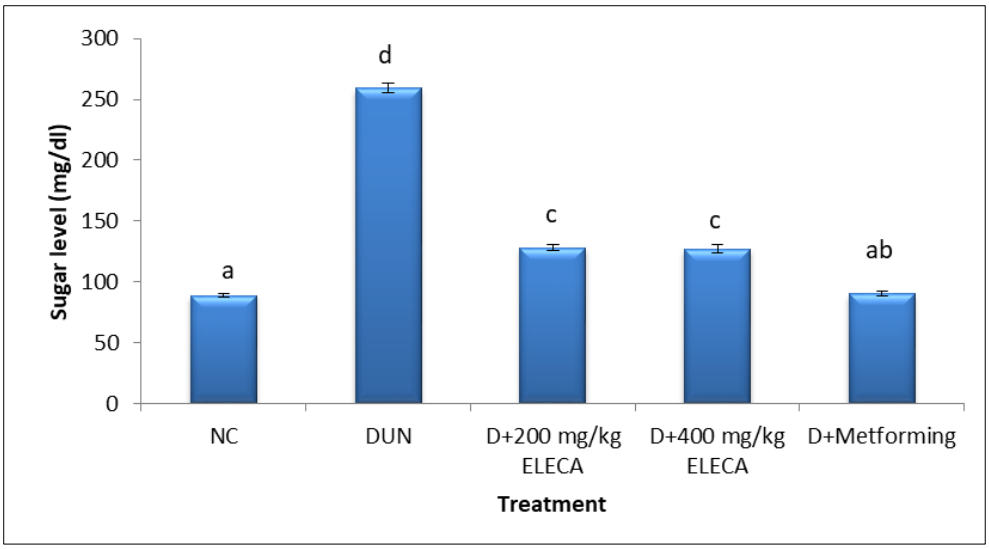
Animals were fasted overnight after which their blood sugar level were determined before induction of diabetes mellitus following intraperitoneal injection of streptozotocine (STZ) dissolved in 0.1 M sodium citrate buffer pH 4.5 at a single dose of 45 mg/kg body weight. 30 minutes after diabetes induction, rats were allowed free access to food and water. 72 h after STZ was administered, the plasma blood glucose level of each animal was determined and rats with fasting blood glucose above 200 mg/dl were considered diabetic and were included in the study [7].

2.6 Histopathological Examination

Liver and pancreas obtained from the experimental animals were fixed for 72 h in 10% formal saline and afterwards dehydrated in graded alcohol before being cleared with two changes of xylene then embedded in paraffin wax. Serial transverse sections of 4-5 micron thickness were prepared with the aid of a microtome, stained with Haematoxylin and eosin (H & E).

2.7 Statistical analysis

Data generated from the study was analyzed using statistic software IBM SPSS Statistics 21 (IBM Corporation,NY, USA). Data were expressed as mean ± standard deviation (SD). The results were considered as significant at P value less than 0.05. Mean values were compared using one way analysis of variance (ANOVA).



Key: NC= Normal control; DNU= Diabetic Untreated; D= Diabetic; ELECA= Ethanol Leaf Extract of Cratevaadansonii

Figure 1 Blood sugar levels of diabetic rats administered with ethanol leaf extract of *Crateva adansonii*

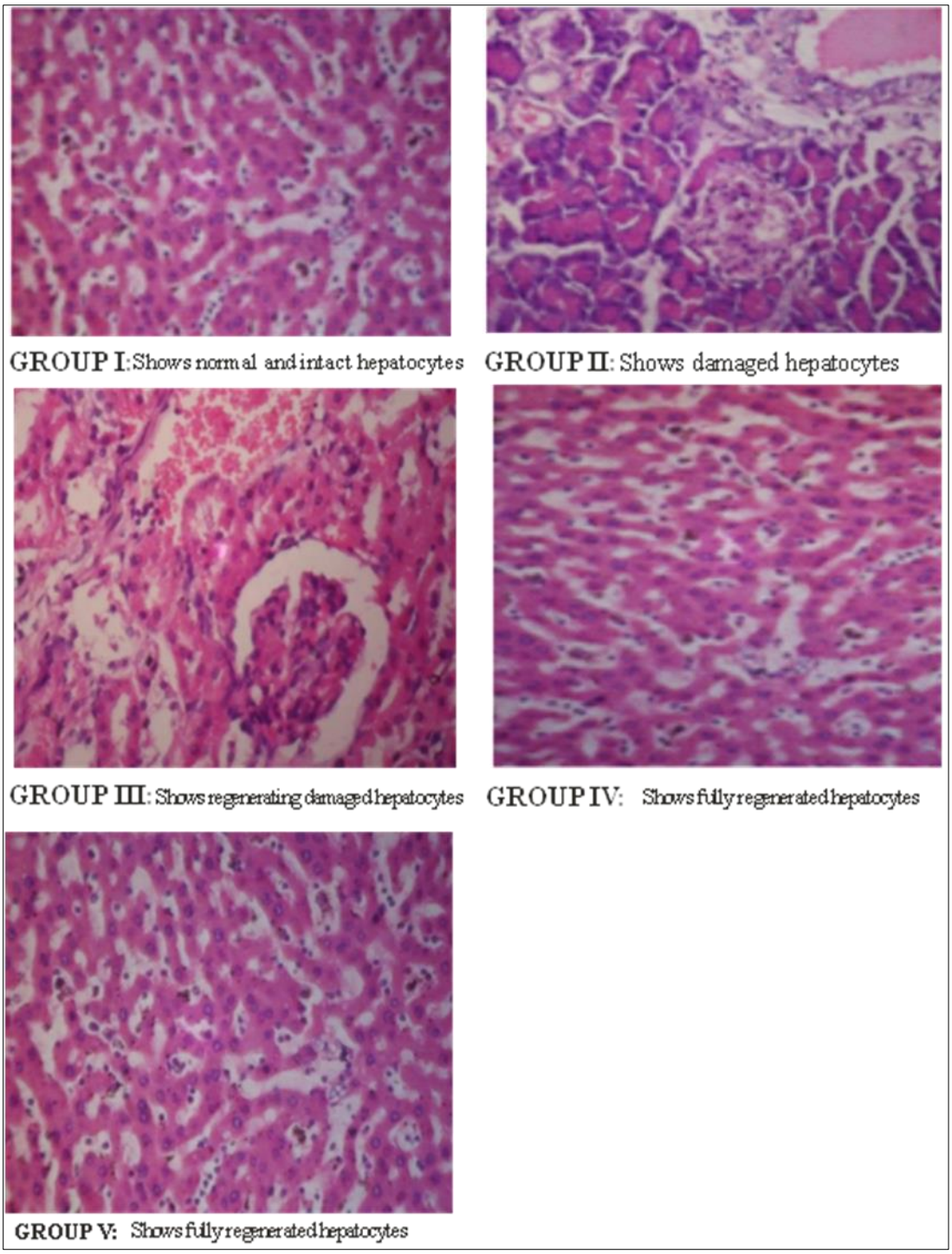


Figure 2 Photomicrographs of the liver of diabetic rats treated with *Crateva adansonii*

3 Results and discussion

Diabetes mellitus is one of the most common causes of liver damage. It has been correlated with the entire spectrum of liver diseases including abnormal levels of serum hepatomarkers, non-alcoholic fatty liver disease, liver cirrhosis and carcinoma [8]. Conventional treatment of diabetes involves sustained reduction of blood glucose levels using different agents such as sulfonylurea and thiazolidinedione [9]. Certain herbal extracts have shown the ability to treat diabetes and prevent the development of its long term complications without causing adverse effects. Intraperitoneal injection STZ caused a marked rise in the blood sugar levels. However, administration of extract caused a significant reduction in the blood sugar levels of rats treated with ethanol leaf extract of *C. adansonii* which although was significantly ($P<0.05$) lower than that reported for the normal control group. The observed reduction in the blood sugar level of diabetic rats

following oral administration of ethanol leaf extract of *Crateva adansonii* could be as a result of enhanced glucose utilization by the peripheral tissues. This result is consistent with the finding of Mukesh and Patil [10] which established that extract of stem bark of *Crateva nurvala* a member of the *capparidaceae* family to which *Crateva adansonii* belongs caused a significant increase in plasma insulin level in treated diabetic rats.

4 Conclusion

The leaf of *Crateva adansonii* has been shown to be endowed with active ingredient(s) with anti-diabetic potential evident by the fact it could reverse a diabetic condition to a healthy one as could be seen in the outcome of this study. However, further research efforts should be geared towards identifing, isolating and characterizing the implicated compound(s).

Compliance with ethical standards

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Disclosure of conflict of interest

Authors hereby declare that no conflict of interest exists.

Statement of ethical approval

Ethical certification was obtained from the Committee on the care and use of Laboratory Animals of the Polytechnics.

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