ejpmr, 2021,8(12), 639-643

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

BIOCHEMICAL, ELECTROLYTES AND KIDNEYS HISTOLOGY OF STREPTOZOTOCIN INDUCE-DIABETIC RATS EXPOSED TO HYDROMETHANOLIC LEAF EXTRACT OF *CNIDOSCOLUS ACONITIFOLIUS* (EUPHORBACAEA)

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Article Received on 21/10/2021 **Article Revised on 11/11/2021** **Article Accepted on 30/11/2021**

ABSTRACT

The aim of this study is to evaluate the biochemical, electrolyte and kidneys histology of Streptozotocin induced diabetic rats exposed to hydromethanolic leaf extract of *CnidoscolusAconitifolius*. Thirty Wistar rats weighing about 190g were randomly divided into six (6) groups of five (5) animals each, diabetes was induced intraperitonially using streptozotocin 60mg/bw. Group 1 served as negative control (non- diabetic) and received normal chow and water *ad libitum,* group 2: served as positive control group (diabetic group) and received no treatment, group 3 received 10mg/kg body weight of glibenclamide; groups 4, 5 and 6 received 100mg/kg bw, 150mg/kg bw and 200mg/kg bw of extracts of *Cnidoscolusaconitifolius* respectively orally for 28days. Animals were sacrificed and blood samples collected for biochemicalanalysis using standard method , data analysed using SPSS version 20 and ANOVA for comparison, the kidneys collected for histopathological studies. The result shows significant ($p<0.05$) decrease in the serum concentration of sodium, chloride, bicarbonate and increases in the concentration of potassium, urea and creatinine. The histopathological study shows alterations in the cytoarchitecture of the renal tissues. Conclusively, the plant have the potency to disrupt the renal functions by altering the biochemical, electrolyte and histology of the kidneys.

KEYWORDS: *CnidoscolusAconitifolius*, biochemical, electrolytes, Streptozotocin.

INTRODUCTION

The efficacy of plant is widely as a result of the presence of phytochemicals contained in different parts of plants. Physiologic effects and potential therapeutic properties of plants are due to various phytochemicals. Some of these plants may display effect on the biochemical, electrolytes and histology of the kidneys of rats in diabetic state exposed to *cnidoscolus aconitifolius.* Herbal plants are being increasingly utilized to treat a large variety of clinical diseases,[1], and some causing very grave danger unknowingly to humanity. So many drugs used today are of plant origin, higher plant source of medicinal compound continue to play a prominent role in the maintenance of human health since cosmogony.[2] Many phytochemicals have been identified as components of food and more are still being studied.[3] Phytochemicals of greater importance are plant steroids, flavonoids, tannins, saponins, glucosides and alkaloids. The Kidneys are paired bean shaped organs that is anatomically situated at the inferior and posterior part of the abdomen. These organs serve several essential regulatory roles in most animals, including vertebrates and some invertebrates. They are essential in the urinary system and also serve homeostatic functions such as the regulation of electrolytes, maintenance of acid-base balance, and regulation of blood pressure (via maintaining salt and water balance). Electrolytes are compounds which when dissolved in a liquid, will conduct electricity. They form positively charged ions (cations) and negatively charged ions (anions). They help to maintain the stability of body fluid. The electrolytes found in the body fluid include sodium, potassium, calcium, chloride, bicarbonate etc. Some of the biochemical parameters are creatinine, urea and bilirubin.[4] Diabetes is a metabolic disorder in which the body does not produce or adequately utilize insulin. It causes a disturbance in carbohydrates, protein and lipid metabolism and some complications. Diabetes mellitus has become a common disease, so prevalent in the world affecting all ages in developed and developing countries.[5] The prevalence of diabetes mellitus in some countries has reached 2-3% of the total populations and in Africa, especially, in Nigeria it is on the increase.[6]

Cnidoscolusaconitifolius is a perennial shrub of the family Euphorbiaceae commonly found in the tropics. It is commonly eaten as a vegetable in soup condiments in south western Nigeria, where it is called 'Iyana apaja'' and ''Efo Jerusalem'', in the Niger Delta is known as '' hospital too far'' due to its multifaceted traditional uses.[7] It has been demonstrated to contain muslin, phenols, saponins, phlobotannins and cardiac glycosides.[8] Chaya consumption has become popular amongst Nigerians and the Hispanic populations in the southern Texas Florida. The nutritional value of *Cnidoscolus aconitifolius* is very rich when compared with other vegetables.[9,10] High fiber content and antibacterial activities of *Cnidoscolus aconitifolius* have been reported.[11] Apart from antibacteria activities, the ameliorative effect of the *Cnidoscolus aconitifolius* on anaemia and increased erythrocyte osmotic fragility induced protein energy malnutrition has also been reported.[12]

Chaya is a food plant; it has been used therapeutically for some ameliorative effect on fasting blood glucose (diabetes)[13], arteriosclerosis, gallstone and high cholesterol. It is also believed that *Cnidoscolus aconitifolius* cleans the circulatory system, stimulates lactation; improve digestion.[14] The effects on reproductive system and liver enzymes have also been studied.[15] Despite gamut of work done on *Cnidoscolus aconitifolius*, scanty literature is available on the biochemical, electrolytes and renal histology of streptozotocin induced diabetic rats exposed to hydromethanolic leaf extracts of *Cnidoscolus aconitifolius*. Hence this study therefore aims at scientifically studying the biochemical, electrolyte and renal histology of streptozotocin induced-diabetic rats exposed to hydromethanolic leaf extract of *Cnidoscolus aconitifolius*.

MATERIALS AND METHODS

Plants materials and Authentification

Fresh leaves of *Cnidoscolus aconitifolius* were obtained from a garden at Seventh day Adventist church, Choba, Port Harcourt, Rivers State and were identified by Dr. N.E Edwin-Wosu of the Department of Plant Sciences and Biotechnology University of Port Harcourt Rivers State, Nigeria with reference number UPH/PSB/015.

Extraction of plants

Fresh leave of *Cnidoscolus aconitifolius* were air dried and pulverized with electric grinding machine into powder weighing 150g. Hydromethanolic (1/4) extraction was obtained using Soxhlet extractor (model no. 3567, Austria). The obtained extract was filtered using Whatmann no 1 filter paper and filtrate was concentrated under reduced pressure in vacuum at 45^0C, using evaporator (Gallenkamp, UK). The residues were dried to a constant weight at 45^0C, the extract stored at 4^0C.

Experimental Design

Thirty six Wistar rats with average weight of 180g were randomly assigned into six groups of six (6) animals each. Group 1: served as negative control (non-diabetic group) and received normal chow and water *ad libitum*, group 2: served as positive control group (diabetic group) and received no treatment, group 3 received 10mg/kg body weight of glibenclamide; a known antidiabetic drug, group 4, 5 and 6 served as experimental groups and received 100mg/kg bw, 150mg/kg bw and 200mg/kg bw of Hydromethanolic leaf extracts of *Cnidoscolus aconitifolius* respectively via oral for 28days after been induced with diabetes using streptozotocin (STZ). Principles of laboratory animals care (NIH publication no 85, revised 119, 1985) were adhered to as well as specific national laws applicable (Iwuji and Nwafor 2014). At the end of the extract administration, animals were anaesthetized using 25% urethane at the dose of 0.6ml/100g bw intraperitoneally; blood sample collected for laboratory determination of serum biochemical and electrolyte level and kidneys harvested for histology.

Induction of Diabetes in Rats

The induction of diabetes using streptozotocin (STZ) was done as described by (Weleh *et.al* 2018).

ELECTROLYTES ASSAY

Determination of sodium (Na^+)

The method is based on modification of those first described by Manuna and Trinder in which sodium is precipitated as the triple salt, sodium magnesium Uranyl acetate, with excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Determination of potassium

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to potassium concentration in the range of 2-9mEq/l.

Determination of Bicarbonate

The method is titrimetric method, where carbondioxide (CO_2) is released from plasma bicarbonate with dilute Hydrochloric acid, excess is titrated against sodium hydroxide using phenyl red as an indicator.

Determination of chloride ion

Chloride ion reacts with Mercurious Thiocyanate to form mercury perchlorate and Thiocyanate which forms a red complex with ferric ions in the presence of Nitric acid.

Determination of Urea

The principle of urea determination is that serum urea is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction.

Determination of Creatinine

The method is colorimetric, creatinine in alkaline solution reacts with picric acid to form a coloured complex whose amount formed is directly proportional to the creatinine concentration.

Histological Analysis

Animal sacrifice was done under aneasthesia with chloroform vapour and the kidneys were harvested and processed for histological assay.

The processed organs from all groups were fixed in 10% formaldehyde, the tissue processing was done using routine paraffin wax processing technique and the prepared 5µ thick section were mounted on slides and stained with H&E.

Statistical Analysis

The data were expressed as the standard error of mean (SEM) and subjected to statistical analysis using Standard Package of Social Science (SPSS) version 20.0, comparison were made between the two controls and the experimental groups using one way analysis of variance (ANOVA). Values were considered statistically significant at ($p<0.05$).

RESULTS

All results obtained from the study were presented in tables and expressed as Mean plus/minus Standard error of Mean. (M±SEM) as below.

Table 1: Effect of *CnidoscolusAconitifolius* on Creatinine, Urea and Electrolytes.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Sodium ion (mmol/l)	137.4±1.5	114. 6 ±1.2[a]	118.4±1.7[a]	123.6±1.5[ab]	127.3±1[ab]	98.5±1.6[ab]
Potassium ion (mmol/l)	5.3±1.4	3.0±3.3[a]	3.4±5.1[ab]	3.8±3.3[ab]	4.4±5.2[ab]	9.2±4.2[ab]
Chloride ion (mmol/l)	57.4±0.9	45.1±0.4[a]	47.8±0.5[a]	44.5±0.8[a]	41.8±0.7[ab]	38.7±0.3[ab]
Bicarbonate ion (mmol/l)	28.0±1.5	20.4±0.9[a]	22.1±0.9[a]	22.8±0.4[a]	19.6±0.5[a]	17.8±0.5[ab]
Urea (mmol/l)	3.9±0.4	10.4±0.6[a]	8.6±0.5[a]	8.3±0.1[a]	7.1±0.3[ab]	2.7±0.9[ab]
Creatinine (µmol/l)	71.6±5.1	54.1±0.9[a]	57.6±0.2[ab]	58.2±4.2[ab]	61.7±2.4[ab]	90.8±3.9[ab]

Data are expressed as Mean $\pm SEM$, n=5, a significantly different from negative control, b significantly different from positive control group ($p<0.05$)

Fig 1: shows photomicrograph of negative control group kidneys, the kidneys manifested normal cyto architecture with glomerular tuft, renal tubules and Bowman's capsule, while fig 2: shows the kidneys of the group 2 animals that were diabetic but not treated and serves as the positive group, it manifested mildly distorted renal morphology with partitioned glomerular tuft, collapsed renal tubules and patent Bowman's capsule. Fig 3 shows the histology of the group 6 animals that received 200mg/kg of *Cnidoscolus Aconitifolius* manifested distorted renal morphology with partitioned glomerular tuft; total collapsed renal tubules and occluded Bowman's capsule.

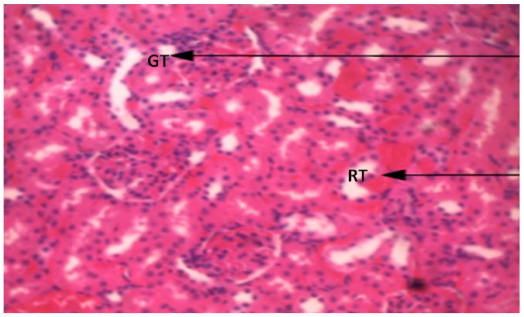

Fig 1: Photomicrograph of kidneys tissue Mag (X400) H&E stain Group 1 control (non-diabetic). Showing normal kidneys architecture, manifesting with glomerular tuft (GT), renal tubules (RT) and Bowman's capsule (BC).

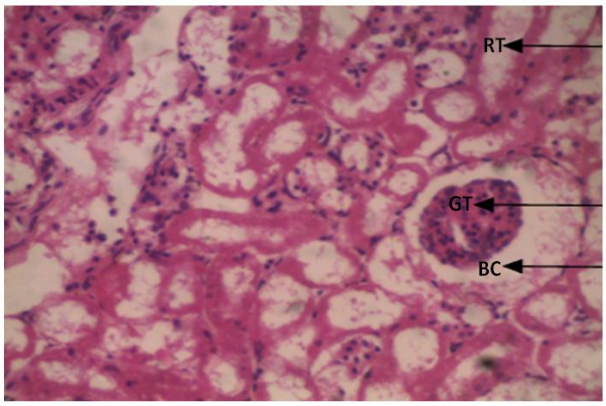

Fig 2: Photomicrograph of kidneys tissue Mag (X400) H&E stain Group 2 (Diabetic+non treatment). Showing mildly distorted kidneys architecture, manifesting with partitioned glomerular tuft (GT), collapsed renal tubules (RT) and patent Bowman's capsule (BC).

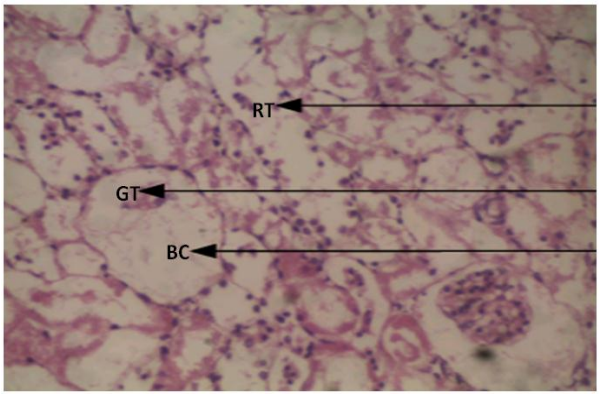

Fig 3: Photomicrograph of kidneys tissue Mag (X400) H&E stain Group 6 (diabetic+ 200mg/kgC.A). Showing distorted kidneys architecture, manifesting with partitioned glomerular tuft (GT), collapsed renal tubules (RT) and occluded Bowman's capsule (BC).

DISCUSSION

Diabetes mellitus is a metabolic disease characterized by numerous disorders that alter the metabolism of carbohydrates, fat and protein. Diabetes and its complications are major problem in the world populations. It has defiled modern medicines like bignuamides, sulphonylureas and thiozolindines.[13]

Alternative medicines, particularly, herbal medicines with rich source of natural products have been widely used for the treatment of diabetes mellitus around the world with less known scientific bases.

This study highlights the effect of hydromethanolic leaf extract of *Cnidoscolus Aconitifolius* on biochemical, electrolytes and histology of the kidneys in a streptozotocin induced diabetic rats. Streptozotocin is a glucosamine nitrosurea compound, after uptake into the body cells is split into glucose and methyl nitrosurea moiety. Methlynitrosurea, due to its alkylating properties, modifies biological macromolecules, fragments DNA and destroys beta cells, causing insulin-dependent ‘’streptozotocin diabetis.[15]

The study reveals alterations in some renal electrolytes, urea and creatinine, electrolytes play pertinent roles in several biological processes which include acid-base balance, controlling of body fluid levels, coagulation, muscle contraction and nerve conduction.[16] Electrolytes homeostasis is maintained within the limits and must be kept at standard level for normal physiological functions.

There were significant decrease in sodium, chloride and bicarbonate ions when compared to the diabetic group and concomitant decrease in the experimental group compared with both negative and positive control groups, there was decrease in potassium in the diabetic group and significant increase in the concentration of serum potassium in the experimental group.

There was significant increase in serum creatinine when compared with the control group; this is similar with the work of Oluwatosin[17], but in variance with works by Achi *et al.*,[18] The difference could be the medium of extraction or concentration of the *cnidoscolus aconitifolius. Cnidoscolus aconititfolius* could have phytochemical components with ability to combine with proteins of the kidneys tubular epithelium, thus, producing Reactive Oxygen Specie. The phytochemical component could also damage juxtaglomerular apparatus due to which renin secretion gets reduced, thus, causing aldosterone reduction which may result in electrolytes imbalance.[15]

The histopathology in the study reveals renal necrosis of the tubular epithelia cells, progressive glomeruloangiosclerosis, disintegration of cellular membrane; this is in variance with a report by[19], who reported that aqueous leaf extract of the plant did not show any degenerative changes in the histology of kidneys. Our result is therefore an indication of renal damage by the hydromethanolic extract of *Cnidoscolus Aconitifolius*. Our study is similar with an earlier report by Oluwatosin[17] who reported that tubular necrosis and presence of protein cast in the tubular lumen and interstitial congestion. The reason for the difference could be as a result of medium used for extraction.

CONCLUSION

Cnidoscolus aconitifolius as a perennial plant is mostly used as vegetables. The study has shown that hydromethanolic extract of *Cnidoscolus Aconitifolius* has potency to alter electrolytes, biochemical and histology of the kidneys and we therefore, recommend that further studies be carried out on the fractionalization and individual study of the phytochemical constituents to ascertain the constituents that is responsible for this deleterious effect on physiological processes and tissue damage.

ETHICAL APPROVAL

Animal Ethic approval was sought to carry out this study. The research was approved by the Ethics Committee of the Centre for Research Management and Development of our University.

COMPETING INTEREST

Authors have declared that no competing interest exists.

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