

n-Hexane leaf fraction of *Jatropha curcas* mitigates hyperglycaemia and hepatic nitric oxide levels in diabetic rats

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ABSTRACT

Hyperglycaemia is considered a primary cause of diabetic vascular complications. Diabetes mellitus is a global health burden that increases the search for herbal hypoglycaemic agents as an alternative to synthetic ones. This research evaluated the effects of n-hexane leaf fraction of *Jatropha curcas* on hepatic nitric oxide levels in streptozotocin-induced diabetic Wistar rats. Thirty-two female rats weighing 100-150g were used. The rats were divided into four (4) groups of eight (8) rats each. Three of the four groups (groups II-IV) were induced with diabetes via intraperitoneal injection of streptozotocin (50mg/kg b.w.) to rats. Group I normal control (NC) received a normal feed. Group II, the diabetic control (DC) received no treatment. Groups III and IV were diabetic treated with 500mg/kg b.w of metformin and 200mg/kg b.w of n-hexane fraction (NHF) of *Jatropha curcas*, respectively. All treatments were administered orally, once daily for twenty-one (21) days. During this period, fasting blood sugar (FBS) was assayed at regular intervals. At the end of the treatment period, the rats were sacrificed and liver tissue samples were collected from the animals and assayed for hepatic concentrations of nitric oxide (NO). The outcome of the result showed a significant ($P < 0.05$) decrease in FBS of the test groups compared with the diabetic control (DC). A further decrease in FBS was observed with the n-hexane fraction (NHF), which was not significant ($P > 0.05$) compared with the normal control (NC). The hepatic nitric oxide level of the diabetic control (DC) was increased, however not significant ($P > 0.05$) compared with the normal control (NC) and the rest of the groups. The n-hexane fraction of *Jatropha curcas* mitigated the hepatic nitric oxide level caused by diabetes, however, it was not significant ($P > 0.05$). This research suggests that the n-hexane fraction of *Jatropha curcas* possesses an anti-hyperglycaemic effect with concomitant reversal of increased nitric oxide level caused by diabetes. The histological evaluation also supports the assertion of its suitability for use in the pharmaco-treatment of diabetes.

Keywords: *Jatropha curcas*, hyperglycaemia, nitric oxide, liver tissue, diabetes mellitus

INTRODUCTION

Diabetes is a serious, chronic, and complex metabolic disorder that affects both the young, old, and its complications may result in liver complications [1]. Diabetes is deemed the most common cause of liver disease and death arising from liver complications [1]. The alarming rise in the population of patients uncounted with this disease is of great concern; thousands are dead and thousands are still dying of this metabolic syndrome [2]. Globally, over 460 million people have diabetes and it is projected to increase to 700 million by 2045 [3]. Chronic hyperglycaemia, the main distinct and diagnostic feature of diabetes is associated with alterations in nitric oxide (NO) levels [4]. Nitric oxide is a gaseous

molecule secreted by the endothelium and a major modulator of endothelial function [5]. It is an important mediator of liver physiology and pathophysiology [6]. NO is synthesized from L-arginine by the family of enzymes called nitric oxide synthases (NOSs) viz. neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS) [7]. However, the endothelial NOS (eNOS) and inducible NOS (iNOS) are the major players in liver biology [6]. The eNOS is membrane-bound, whereas iNOS is predominantly found in the cytosol. The eNOS-derived NO is protective against liver disease, whereas iNOS-derived NO is deleterious [6]. Under many physiological conditions, iNOS produces large amounts of NO,

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which is a major source of reactive nitrogen species (RNS), particularly peroxynitrite (ONOO-), which can damage cellular molecules such as DNA, lipids, and proteins and alter the structure and function of target proteins as well [6, 8]. NO acts as a pleiotropic intracellular messenger, exerting various biological actions under both physiological and pathological conditions [4]. Nitric oxide exhibits both beneficial and detrimental effects, while low levels are beneficial for several physiological and cellular functions, high levels could result in the production of secondary toxic compounds capable of damaging many biological molecules leading to tissue injury [9]. The long-term effects of diabetes mellitus include cellular damage, inflammation, and malfunction of different organs [9]. The complications of diabetes are divided into macrovascular complications that include coronary artery diseases, peripheral vascular disease, stroke, and microvascular complications, which also include diabetic nephropathy, retinopathy, and neuropathy [4]. Among all complications, endothelial dysfunction is a common problem in all patients with diabetes. Endothelial cells secrete different mediators such as vasodilators like nitric oxide and vasoconstrictors like endothelin-1 [10]. Hyperglycaemia and other metabolic changes may lead to the impairment of nitric oxide (NO)

AIM OF THE STUDY

This research evaluated the effect of n-hexane leaf fraction of *Jatropha curcas* on hepatic nitric oxide levels in

production [11]. Impairment of endothelial function in type II diabetes mellitus (T2DM) patients ultimately leads to cardiovascular diseases [12]. Thus, endothelial dysfunction is the early feature of cardiovascular complications in T2DM [13]. Insulin regulates nitric oxide production, which mediates to a large extent the vasodilation, anti-inflammatory, and antithrombotic properties of a healthy endothelium [14]. Diabetes or hyperglycaemia results in an alteration of vascular homeostasis changing NO levels [14], by creating an imbalance between NO bioavailability and accumulation of ROS as well as RNS, resulting in endothelial dysfunction [12]. The maintenance of vascular homeostasis by regulating the NO balance that diabetes alters is an important research undertaking. It is anticipated that the use of medicinal plants with numerous medicinal and health benefits as *Jatropha curcas* might achieve that aim. *Jatropha curcas*, a flowering plant in the spurge family, Euphorbiaceae, indigenous to America's tropics, particularly Mexico and Central America, but now, extensively cultivated in both tropical and subtropical regions of the world are a medicinal plant with anti-diabetic properties [15, 16]. Medicinal plants with anti-diabetic properties might be suitable for addressing the alteration of nitric oxide levels associated with diabetes.

streptozotocin-induced diabetic Wistar rats.

Materials and Methods

Collection of plant material

Fresh leaves of *Jatropha curcas* were collected from Okuku, in Yala Local Government Area of Cross River State, Nigeria. Mr. Frank Apojeye at the

Herbarium Unit, Department of Botany, University of Calabar, Calabar, identified and authenticated the plant with ID No: 67.

Preparation of whole leaf extract

The fresh leaves of *J. curcas* were air-dried at room temperature for one month. After which, the dried leaves were pulverised and 100 g was extracted in 400 mL of absolute ethanol (BDH) at room temperature via maceration. The suspension was agitated and allowed to stand for 72 h, after which it was first

filtered with a cheesecloth, and later with Whatman No.1 filter paper (24 cm). The filtrate was evaporated using a dry air oven (ES-4620, Ecoskhim Ltd, Ecos group of companies, Saint-Petersburg, Russia) at 40 °C until there was no further loss of solvent, giving a constant weight.

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This semi-solid extract was then used for

fractionation to obtain n-hexane fraction.

Liquid-Liquid fractionation of the whole extract

Briefly, 10g of ethanol leaf extract in a separating funnel was solubilised with an aliquot of ethanol (the extraction solvent), n-hexane was added and agitated vigorously. This thoroughly agitated suspension was allowed to stand until two clear visible layers (fractions) were separated based on the differential densities of the two solvents: the denser ethanol fraction (residue) beneath and the less dense n-hexane fraction above. The fraction was collected, and the whole cycle was repeated until all n-hexane-

soluble components were collected and pooled into a separate beaker and labelled, leaving the residue. Accordingly, the whole extract was separated into n-hexane fraction and the ethanol extract residue. The n-hexane fraction was oven-dried at 40 °C to dryness, yielding a semi-solid n-hexane fraction (NHF), which was stored at 4 °C for the animal experiments. In the animal experiments, the n-hexane fraction (NHF) was reconstituted in 3% Tween-80 before administration via oral gavage.

Animals

Thirty-two (32) female Wistar rats weighing 100-150 g were obtained from the animal house of the Department of Medical Biochemistry, Cross River University of Technology, Okuku Campus, and kept in well-ventilated laboratory

cages and feed tap water and standard rat pellets *ad libitum*. The animals were handled in line with ethical guidelines and approved by the Faculty of Basic Medical Sciences Ethical Committee, CRUTECH.

Induction of diabetes

Twenty-four (24) of the rats were fasted overnight and induced with diabetes by intraperitoneal administration of 50 mg/kg BW of streptozotocin (STZ) reconstituted in cold physiological saline. On the third day, approximately 72 h post-STZ injection, fasting blood sugar (FBS) level was determined using a One-

touch ACCU-CHEK Advantage glucometer (Model - GB 13117699, Roche Diagnostics, Mannheim, Germany), using blood obtained from the tail vein puncture. Rats with FBS \geq 200 mg/dL and \leq 450 mg/dL were considered diabetic and chosen for the trial.

Design of experiment and treatment plan

Thirty-two rats comprised 24 diabetic of 8 rats each and 8 non-diabetic rats were divided into four groups and treated, thus:

Group IV: Diabetic rats treated with 200mg/kg BW of n-hexane fraction (NHF) of *Jatropha curcas*, reconstituted with 3% Tween-80.

Group I: Normal control (NC); non-diabetic rats, given normal saline in 3% Tween-80 (reconstitution solvent).

The extract and fractions were administered via oral gavage once per day for 21 days. Subsequently, diethyl ether (5%) was used to anaesthetise the animals after an overnight fast. The liver was surgically excised under aseptic conditions and used for tissue nitric oxide determination.

Group II: Diabetic control (DC); non-treated diabetic rats, given normal saline in 3% Tween-80 (reconstitution solvent).

Group III: Standard control; diabetic rats treated with 500mg/kg BW of Metformin (MF) reconstituted with 3% Tween-80.

Determination of serum glucose

The fasting blood sugar (FBS) method was used to determine serum glucose levels using a One-touch ACCU-CHEK Advantage glucometer (Model - GB 13117699, Roche Diagnostics, Mannheim, Germany), using

blood obtained from the tail vein puncture. This procedure was repeated at intervals of three days until the final day of the experiment when the last reading for FBS was taken before sacrifice.

Determination of Nitric Oxide

Nitric Oxide was determined using the modified method of Matthew *et al.* [17].

Preparation of liver samples for histological examination

The liver of the rats was quickly removed, washed in normal saline, and fixed in 10% formal calcium. The organs were processed as described by Mbaka *et al* [18]. Sections of 4µm of the organs were

cut using a microtome stained with haematoxylin and eosin. This was visualized using the light microscope at x 100 and x 400 magnifications and the photomicrographs were captured.

Statistical Analysis

Data obtained was analysed using SPSS with one-way ANOVA and $P < 0.05$ was

considered statistically significant. Data are expressed as the mean \pm SEM.

RESULTS

The results of the n-hexane fraction of *Jatropha curcas* on hyperglycaemia and hepatic nitric oxide of diabetic Wistar rats

are given in Table 1, while the liver tissue histology is presented in Plate 1.

Results of diabetic fasting blood sugar (FBS) and hepatic nitric oxide of Wistar rats treated with n-hexane fraction of *Jatropha curcas*

The results of the initial FBS, Final FBS, and hepatic nitric oxide, respectively, of diabetic untreated and treated Wistar rats are given in Table 1. The outcome of the result after 21 days of administration shows a significant ($P < 0.05$) decrease in FBS of the test groups compared with the diabetic control (DC). A further decrease in FBS was observed with the n-hexane fraction (NHF), which was not significant

($P > 0.05$) compared with the normal control (NC). The hepatic nitric oxide level of the diabetic control (DC) was increased, however not significant ($P > 0.05$) compared with the normal control (NC) and the rest of the groups. The n-hexane fraction of *Jatropha curcas* could reverse hepatic nitric oxide level caused by diabetes, however, it was not significant ($P > 0.05$).

Table 1: Fasting blood sugar (FBS) and hepatic nitric oxide concentrations of diabetic untreated and diabetic treated rats with n-hexane fraction of *J. curcas*

Group	Initial FBS (mg/dL)	Final FBS (mg/dL)	Nitric oxide (mol/L)
I (NC)	66.6 \pm 3.56 ^a	75.8 \pm 1.8 ^a	0.34 \pm 0.01 ^a
II (DC)	261 \pm 15.62 ^b	264 \pm 14.31 ^b	0.35 \pm 0.01 ^a
III (MF)	271 \pm 15.62 ^b	86.6 \pm 4.57 ^a	0.36 \pm 0.01 ^a
IV (NHF)	320 \pm 23.75 ^c	58.8 \pm 7.75 ^a	0.33 \pm 0.00 ^a

Values are the mean \pm SEM (n=5). Values with different superscripts (a, b, c) along the column are statistically significant ($P < 0.05$). Legend: NC=normal control, DC=diabetic control, MF=metformin, NHF= n-hexane fraction.

Histology of the liver of diabetic-untreated and diabetic-treated rats (shown in plate 1)

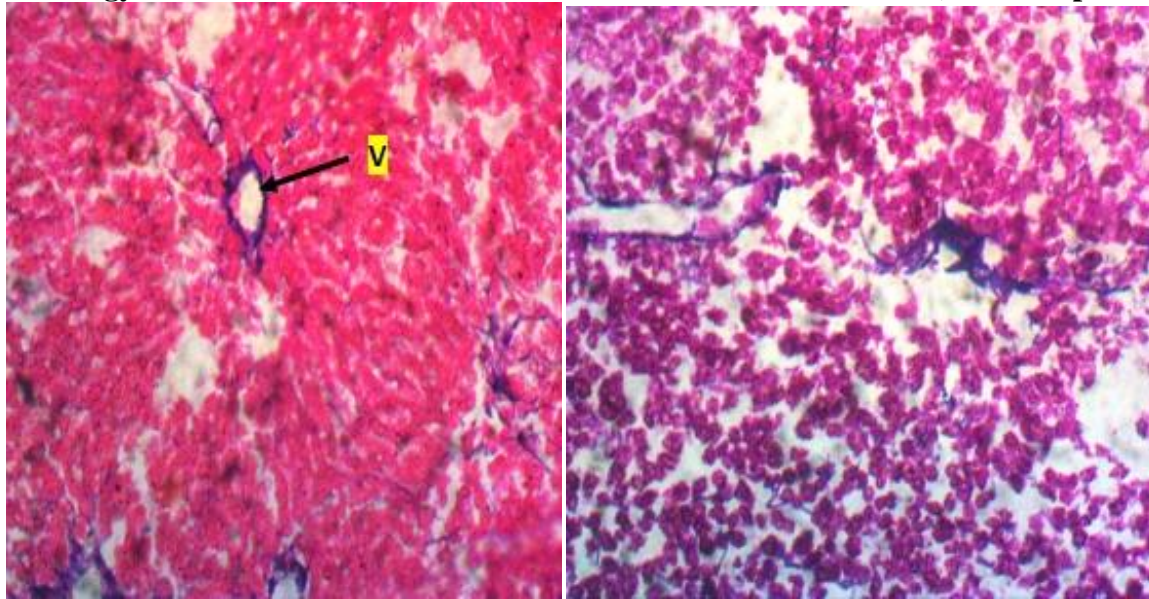


Plate 1 (a)

Plate 1(a) is a photomicrograph (H & E x400) showing the normal cytoarchitecture of the liver. The central vein (V) is interspersed with metaplastic hepatic tissue.

Plate 1(b)

Plate 1(b) is the photomicrograph (H & E x400) of the liver of diabetic untreated rats showing moderate diffused hepatocellular atrophy. Hepatic cells are showing early fibrotic changes, thereby leading to loss of hepatic cells.

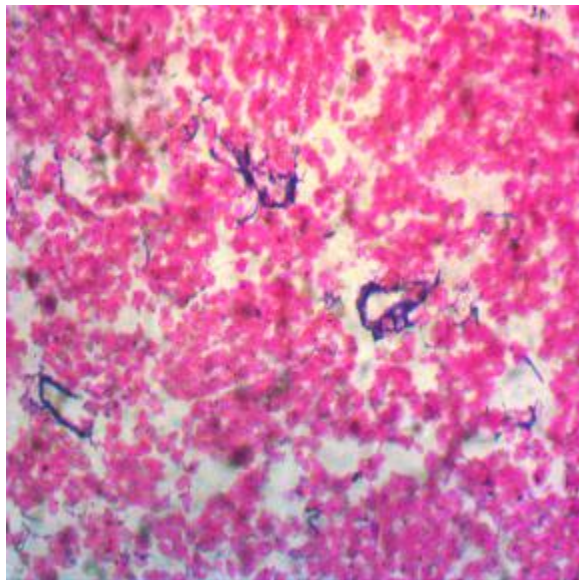


Plate 1 (c)

Plate 1 (c) is the photomicrograph (H & E x400) of the liver of metformin-treated rats showing moderate diffused hepatocellular atrophy. The microstructure shows loss of hepatic cells. Plate 1 (d) is

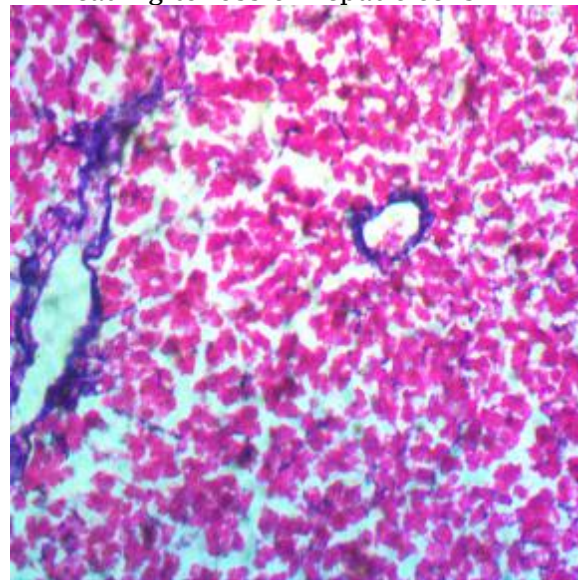


Plate 1 (d)

the photomicrograph (H & E x400) of the liver of n-hexane fraction of *J. curcas* treated rats showing moderate diffused hepatocellular atrophy with dilated sinusoids.

DISCUSSION AND CONCLUSION

DISCUSSION

In the current investigation, the study of the mitigatory effect of n-hexane fraction of *Jatropha curcas* on hyperglycaemia and hepatic nitric oxide level in diabetic Wistar rats provides some useful insight on the antidiabetic potency of *Jatropha curcas*. Diabetes is a degenerative metabolic disease condition that is characterized by uncontrolled blood sugar levels over a prolonged period, due either to the body not producing enough insulin or because the cell does not respond to the insulin produced [1]. There is chronic hyperglycaemia in diabetes and is associated with long-term damage, dysfunction, and failure of different organs, especially the liver, kidney, heart, eyes, nerves, and blood vessels [4, 9]. High blood sugar produces classic symptoms of polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (excessive hunger) [1]. Chronic hyperglycaemia, the main distinct and diagnostic feature of diabetes is associated with alterations in nitric oxide (NO) levels [4]. Nitric oxide is a gaseous molecule secreted by the endothelium and a major modulator of endothelium function [5]. High levels of nitric oxide may have detrimental effects on tissues, while low levels are beneficial for several physiological and cellular functions [9]. The n-hexane fraction of *Jatropha curcas* used in this work showed a strong anti-hyperglycaemic effect as there was a significant reduction of serum glucose level within 21 days of administration. The n-hexane fraction is imbued with strong antihyperglycaemic potential to reverse high blood glucose levels better than the standard drug (metformin). It can

This research suggests that the n-hexane fraction of *Jatropha curcas* possesses an anti-hyperglycaemic effect with concomitant mitigation of increased nitric

also be assumed that the leaf of *Jatropha curcas* may have caused the regeneration of the beta cells to produce insulin as the glucose level of the diabetic treated rat reduced, while that of the untreated remained high and unchanged. The result obtained in the course of this research also shows that the hepatic nitric oxide level of the diabetic control was increased. This outcome corroborated the findings of Adela, *et al.* [9] who reported increased nitric oxide levels in diabetics as a result of hyperglycaemia. High levels of nitric oxide are not only detrimental but may affect cellular proteins structure and function [6]. Though in the current research, the increase of nitric oxide in the diabetic rats was not significant but the n-hexane fraction of *J. curcas* appeared to have mitigated the increased nitric oxide by diabetes to normal. It is likely too that the extract may have encouraged the production of endothelial NO, which is protective and reduced inducible NO which is deleterious and must have been produced more in the diabetic state. Further research will be needed to buttress that. The properties exhibited by the n-hexane fraction are comparatively better than the standard drug (metformin), hence the need to consider its addition in the potential use for pharmaco-treatment of diabetes-induced nitric oxide imbalance. The histological evaluation showed little alteration in the cytoarchitecture of the liver, hence confirming the suitability of the n-hexane fraction of *Jatropha curcas* for treating diabetes-induced nitric oxide alteration.

CONCLUSION

oxide levels caused by diabetes. The histological evaluation also supports the assertion of its suitability for use in the pharmaco-treatment of diabetes.

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