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(RESEARCH ARTICLE)

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Assessment of the effect of ethanolic extract of *Justicia secunda* on blood glucose levels and liver function in alloxan-induced diabetic Wistar rats

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Abstract

Diabetes mellitus (DM) encompasses a range of syndromes characterized by elevated blood glucose levels and disturbances in the metabolism of lipids, carbohydrates, and protein. The liver is one of the many organs of the body that could be affected by DM. This study evaluated the effect of ethanolic leaf extract (ELE) of *Justicia secunda* on blood glucose level and liver function of alloxan-induced diabetic wistar rats. Twenty-five (25) female wistar rats weighing between 150-200g were divided into five (5) groups of (5) rats per group. Group N (Normal rats) received feed and water only, Group Un D (Diabetes untreated) received 150 mg/kgBW of alloxan monohydrate (AMH) intraperitoneally (IP), Group Met received 150 mg/kgBW of AMH IP + 30 mg/kgBW of Metformin while Group 200 mg/kg + D and Group 400 mg/kg + D received 150 mg/kgBW of AMH IP, + 200 mg/kgBW and 400 mg/kgBW of ELE of *Justicia secunda* leaf extract respectively. Administrations were given orally for fourteen (14) days. Data obtained were analyzed using one-way ANOVA (GraphPad Prism version 9.5.1 software package) and values were considered significant at $p \le 0.05$. The findings revealed that ELE of *Justicia secunda* exhibited a significant ($p \le 0.05$) dose-dependent reduction in blood glucose levels when compared to the diabetic control group. Also, there were lower alanine aminotransferase and alkaline phosphate levels, which may signify the hepatoprotective effect of *Justicia secunda*. In conclusion, ELE of *J. secunda* could be beneficial in the management of diabetes mellitus, and improve liver function in wistar rats.

Keywords: Diabetes mellitus; Justicia secunda; Blood glucose; Liver function

1. Introduction

Diabetes mellitus (DM) is the collective term for heterogeneous metabolic disorders whose main finding is chronic hyperglycemia. The cause is either due to disturbed insulin secretion by the pancreas, or disturbed insulin effect, or both ¹. According to the 2015 International Diabetes Federation report, around 415 million people worldwide suffer from this disease, and it is estimated to increase to 642 million in 2040 ^{2,3,4}. The two most common types of diabetes are type 1 and 2. Type 2 diabetes (T2DM) has a high prevalence, accounting for about 90% – 95% of cases ^{4,5}.

The liver is by far, the most important metabolic organ with essential roles in regulating homeostasis and mediating glucose and lipid metabolism. Metabolic activities of the tissue are precisely controlled by the actions of metabolic substrates, including free fatty acids (FFAs) and hormones ^{6,7}. T2DM patients usually suffer from a chronic liver condition called non-alcoholic fatty liver disease (NAFLD). It is characterized by steatosis, i.e. ectopic fat storage in

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hepatocytes and subsequent insulin resistance ^{7,8}. Overt DM is more prevalent in patients with the liver disease: chronic hepatitis C, than in patients with other liver diseases ⁹.

Liver function can be accessed by determining the level of several substances made by the liver, in blood samples. These substances are largely proteins and enzymes, and they include total protein (i.e. albumin and globulin), albumin (a protein made in the liver), enzymes such as alanine transaminase (ALT), aspartate amino transferase (AST), alkaline phosphatases (ALP), gamma glutamyl transferase (GGT), and lactate dehydrogenase (LDH) all made largely in the liver, as well as by measuring billirubin (a waste product from the breakdown of old red blood cells and removed by the kidney), or prothrombin time (PT), which is the time it takes for blood to clot.

Justicia secunda, commonly known as "blood root" or "ogwu obara" and "obarayim" in some South Eastern Nigerian villages and "obara bundu", "asindiri" or "ohowaazara" in the Niger Delta region of Nigeria ¹⁰. It is also found in various regions around the world, and has a long history of traditional use in treating various ailments, including diabetes. Numerous studies have investigated the therapeutic potential of *Justicia secunda* in managing diabetes and its associated complications. Studies have revealed that the ethanolic extract (ELE) of *J. secunda* leaves possesses antiinflammatory ¹¹, antinociceptive and antioxidant properties ^{12,13}, antihypertensive ¹⁴, superoxide anion radical scavenging ¹⁵, antioxidant ¹⁶, antimicrobial, antiviral and hematinic potentials ¹⁷

2. Methods

Location of Study: This study was carried out in the Animal House of Department of Physiology, Faculty of Basic Medical sciences, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus.

Materials: Standard plastic cages and water can, Electronic weighing balance (M-Metallar M311), Animal weighing balance, (Camry LB11), Automatic Water distiller (SZ-1 Search Tech Instrument), Gluco Dr. Auto Glucometer, Glucose strips (Gluco Dr. Auto strips), Chloroform (JHD Chemicals, Guangdong China), Hypodermic sterilize syringes, Refrigerator, Stopwatch, Alloxan (Sigma Aldrich, USA), 10% Formalin, Normal saline, Cotton wool (KENS LINT, Benin City, Nigeria), Oral cannula, Dissecting kits, Metformin, Measuring cylinder (MINGHE), S. Pyrex Beakers (Techmel, USA), Supermax latex medical hand gloves (Selangor, Malaysia), Organ bottle, EDTA bottle, Standard pellet vital feed (grower), Lancet, Centrifuge 90(1) (Alpin Medical, England), Spectrophotometer (Shanghai, Yoke Instrument Co., Ltd. China), and Ethanolic leaf extract of *Justicia secunda*.

Collection and preparation of plant extract: *Justicia secunda* leaves were collected from local bushes in Okofia, Otolo Nnewi, Anambra State. The leaves were identified and authenticated by the Botany department of Nnamdi Azikiwe University, and a herbarium number of NAUTH-203^B was assigned.

Extraction Procedure: The leaves of *Justicia secunda* were washed thoroughly using distilled water and cut into tiny pieces to hasten the drying process, The leaves were thereafter, air dried for a period of three (3) weeks before it was grounded into powder using a sterile electric blender. Two hundred and fifty grams (250g) of the powdered sample was macerated in two liters (2L) of ninety–eight percent (98%) absolute ethanol, and allowed to soak for seventy-two (72) hours with intermittent hand shaking. The mixture was sieved using a standard sieve. The filtrate was allowed to settle down for twenty –four (24) hours, and then the ethanol was decanted off while the residue was dried using an extract run bag (99% sodium oxide) made in England. The extract was again air dried and stored in a refrigerator to be used for further studies.

Experimental Design and Animal Grouping: Twenty five (25) healthy wistar rats weighing between 150g-200g were obtained from the Animal House of the Department of Physiology, Faculty of Basic medical science, College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus. The wistar rats were put into different cages, and were kept under close observation in these standard cages at temperature between 25°c to 27.5°c. The animals had free access to normal laboratory rat chow (growers) and clean water. They were acclimatized for a period of fourteen (14) days. After the acclimatization period, the animals were injected with alloxan dissolved in sterile normal saline at a dose of 150 mg/kgBW intraperitoneally (IP). After 72 hours of injection, animals with fasting blood glucose level (FBGL) at or above 126mg/dL (7.0mmol/L) were considered diabetic. The rats were weighed before and after the administration of AMH and thereafter weighed once weekly on Fridays before feeding.

The animals were randomly separated into five (5) groups of five (5) animals each. The administration of ELE of *Justicia* secunda was done as follows:

- Group N: Normal rats (feed and water ad libitum)
- Group Un D: (Diabetes untreated)
- Group Met: (Diabetes + 30 mg/kg of standard drug Metformin)
- Group 200mg + D: (Diabetes + 200 mg/kg of ELE of Justicia secunda)
- Group 400mg + D: (Diabetes + 400 mg/kg of ELE of Justicia secunda)

All administrations were done orally using an oral cannula. At the end of the fourteen-day treatment period with *J. secunda*, animals were anaesthetized using chloroform in an enclosed container for two minutes. After that, blood samples were collected from the animals using a non-heparinized capillary tube via ocular puncture. The blood samples were put in well-labeled EDTA containers for further analysis.

Determination of Blood Glucose level: Blood glucose levels were estimated using Gluco Dr. Auto glucometer by puncturing the tail of the animals with a lancelet and then placing it on the Gluco Dr. Auto strip. Blood glucose level was read off the screen and documented.

Determination of Liver Function: The blood samples collected were centrifuged, stored, and the serum was used to assess the levels of alkaline phosphate (ALP), alanine amino transferase (ALT), and aspartate amino transferase (AST), which were analysed spectrophotometrically with Randox assay kits using the procedure described by Reitman and Frankel ¹⁸.

Statistical Analysis

Data obtained were analyzed using one-way ANOVA (GraphPad Prism version 9.5.1 Software Package), and p values less than or equal to 0.05 ($p \le 0.05$) were considered to be statistically significant.

3. Results

Figure 1 shows a significant dose-dependent reduction in fasting blood glucose levels in the groups treated with ELE of *Justicia secunda* (i.e. groups 200mg +D and 400mg +D), when compared to the diabetes untreated group (Group UN D).



Figure 1 Effect of Ethanolic leaf extract of *Justicia secunda* on Blood Glucose Level in Alloxan-induced Diabetic Wistar Rats

Figure 2 shows that ALT was significantly higher in the untreated diabetes (Un D) group compared to the normal rats (Group N). There was also significantly lower ALT levels in the group administered 400 mg/kgBW ELE of *Justicia secunda*, when compared to the untreated diabetic group ($p \le 0.0150$). Again, a significantly lower level of ALT was seen

in the group treated with Metformin (Met) when compared to the untreated diabetic group ($p \le 0.0190$). There was a significantly higher level of ALT in the diabetic group treated with 200 mg/kgBW of the extract (200 mg/kgBW + D) when compared to the diabetic group treated with 400 mg/kgBW of the extract (400 mg/kgBW + D) ($p \le 0.0294$).



Figure 2 Effect of Ethanolic leaf extract of Justicia secunda on ALT Levels in Alloxan-induced Diabetic Wistar Rats

Figure 3 shows that there was no significant difference in AST levels across all the groups.



Figure 3 Effect of Ethanolic leaf extract of Justicia secunda on AST Levels in Alloxan-induced Diabetic Wistar Rats

Figure 4 shows that ALP was significantly higher in the untreated diabetes (Un D) group compared to the normal rats (Group N). There was also a significantly lower ALP level in the group administered 400 mg/kgBW ELE of *Justicia secunda* when compared to the untreated diabetic group ($p \le 0.0052$). A significantly lower level of ALP was seen in the group treated with Metformin (Met) when compared to the untreated diabetic group ($p \le 0.0052$). A significantly lower level of ALP was seen in the significantly higher level of ALP in the diabetic group treated with 200 mg/kgBW of the extract (200 mg/kgBW + D) when compared to the diabetic group treated with 400 mg/kgBW of the extract (400 mg/kgBW + D) ($p \le 0.0247$).



Figure 4 Effect of Ethanolic leaf extract of Justicia secunda on ALP level in Alloxan-induced Diabetic Wistar Rats

Table 1 Effect of Ethanolic leaf extract of Justicia secunda on ALT, AST, and ALP levels in Alloxan-induced Diabetic WistarRats

Groups	ALT (µ/L)	p value	AST (µ/L)	P value	ALP (µ/L)	P value
Ν	11.43±0.97	0.0119	12.17±0.95	0.1854	80.00±6.08	0.0008
Un D	12.13±0.72		12.13±1.60		97.33±12.0	
Met	12.05±0.25		12.15±1.25		103.50±1.50	
200mg + D	11.90±0.50		10.90±0.50		92.00±2.00	
400mg + D	09.97±0.59		10.40±0.53		72.67±3.51	

4. Discussion

Diabetes mellitus is one of the most common chronic diseases associated with hyperglycemia, hyperlipidemia, comorbidites such as obesity, hypertension, and damage to organs such as the eyes, pancreas, kidneys, and liver. T2DM patients usually suffer from non-alcoholic fatty liver disease (NAFLD), characterized by steatosis and subsequent insulin resistance ^{7,8}.

The present study investigated the effect of two (2) doses of ethanolic leaf extract of *Justicia secunda* (200 mg/kgBW and 400 mg/kgBW) on blood glucose levels and liver function in alloxan-induced diabetic wistar rats, and also in comparison to the standard drug, Metformin (30 mg/kgBW). The study indicates that ELE of *J. secunda* significantly reduced the elevated fasting blood glucose level (FBGL) of the wistar rats in a dose-dependent manner, when compared to the untreated diabetic control group (Un D). This study finding is similar to a study reported by Martinez-Mora *et al.* ¹⁹. The possible mechanism by which *Justicia secunda* brings about its hypoglycemic action may be via four (4) possible ways: potentiating insulin effect in the plasma, increasing pancreatic secretion of insulin from β -cells of islets of Langerhans, increasing the release of insulin from proinsulin, or by increasing the peripheral utilization of glucose.

Liver function can be accessed by determining the blood level of several enzymes produced in the liver such as alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatases (ALP), gamma glutamyl transferase (GGT), etc. For this study, liver function was assessed by measuring levels of ALT, AST, and ALP enzymes in the blood.

The result revealed that there was a significantly lower level of ALT and ALP levels in the Wistar rat group administered 400 mg/kgBW ELE of *J. secunda* (400 mg/kgBW + D), when compared to other groups, which may signify the hepatoprotective effect of *Justicia secunda* at high levels. This study contradicts the study findings by Dutta *et al.* ²⁰. There was no significant difference in AST level when the treated groups were compared to normal rats (Group N).

5. Conclusion

The study findings reveal that *Justicia secunda* is effective in managing hyperglycemic conditions associated with DM, and could serve as an alternative in managing the condition. Also, *Justicia secunda* showed a promising effect in lowering the chances of liver damage, as it improved ALT and AST levels. However, it was not effective on ALP at the same doses. Therefore, studies should be conducted to certify the effect of higher doses of *J. secunda* on ALP levels and other enzymes.

Compliance with ethical standards

Acknowledgement

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Disclosure of conflict of interest

There is no conflict of interest.

Statement of ethical approval

Ethical approval was obtained from the Faculty of Basic Medical Science Ethical Committee, Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikwe University, Nnewi Campus. Rat handling and treatment conform to the rodent handling and restraint (JoVE science education) manual.

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