World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(RESEARCH ARTICLE)

Check for updates

Evaluation of the effect of ethanolic extract of *Justicia Secunda* on oxidative stress and renal function in alloxan-induced diabetic Wistar Rats

Chidinma Winifred Chukwukaeme [1,*], Ada Ak Akwari [2], Chinonye Ejekwurunwa [1], Mary Chioma Igbokwe [2], Chamberlin Nwankwo [1], Ifeanyi John Okoronkwo [1], Kester Eluemunor Nwaefulu [1], Vincent Umeanozie [1] and Jeffrey Ndubueze [1]

[1] *Department of Human Physiology, Faculty of Basic Medical Sciences, College of Health Sciences, Nnewi Campus, Nnamdi Azikiwe University, Nigeria.*
[2] *Department of Human Physiology, Faculty of Basic Medical Sciences, David Umahi Federal University of Health Sciences, Uburu, Ebonyi State, Nigeria.*

World Journal of Biology Pharmacy and Health Sciences, 2025, 22(01), 531-538

Publication history: Received on 04 March 2025; revised on 20 April 2025; accepted on 22 April 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.22.1.0405

Abstract

Hyperglycemia, oxidative stress, and complications of diabetes are closely linked together. The main characteristic condition in diabetes mellitus (DM) is oxidative stress, and increased levels of oxidative stress markers such as Malondialdehyde (MDA) are reported in diabetes. The increasing prevalence of type 2 diabetes (T2DM) worldwide has led to a concomitant rise in the prevalence of diabetes-related complications, including renal disease. Protection of cells against oxidative stress is through antioxidant enzyme activities of SOD, CAT, and peroxidases. *Justicia Secunda* Vahl is a medicinal plant that originates from South America. This study was aimed at determining the effect of ELE of *J. secunda* on oxidative stress, antioxidant levels, and renal function of diabetic Wistar rats. The animals were grouped into five (5) groups of five (5) rats per group. Group N served as the control and received food and water only. Group Un D served as the untreated diabetic group while groups Met, 200mg/kg + D, and 400mg/kg + D were induced with diabetes using AMH two weeks prior to the experiment, and then treated with 30mg/kgBW of Metformin, 200mg/kgBW ELE of *J. secunda* extract, and 400mg/kgBW ELE of *J. secunda* extract, respectively, for fourteen (14) days. Data were analyzed using one-way ANOVA (GraphPad Prism version 9.5.1 software package) and results were considered significant at $p \leq 0.05$. The study revealed that 400mg/kgBW ELE of *J. secunda* drastically reduced MDA levels. Both doses significantly increased SOD and CAT, thereby increasing antioxidant activity. However, the standard drug, Metformin was more effective than *J. secunda* in all aspects except in improving SOD level. There were no significant changes in urea and creatinine levels. The study therefore indicates that *J. secunda* may be beneficial in the management of DM and diseases related to oxidative damage. Therefore, it can be used as an alternative drug in managing these conditions.

Keywords: *Justicia Secunda*; Diabetes Mellitus; Oxidative Stress; Renal Function

1. Introduction

Diabetes mellitus (DM) is the most common endocrine disorder that affects more than 100 million people worldwide (6% population). It is caused by a deficiency or ineffective production of insulin by the pancreas, which results in higher concentrations of glucose in the blood. It is found to damage many body systems, particularly blood vessels (diabetic vasculopathy), eyes (diabetic retinopathy), kidneys (diabetic nephropathy), heart (diabetic cardiomyopathy), and nerves (diabetic neuropathy) [1].

* Corresponding author: Chidinma W. Chukwukaeme.

Diabetic nephropathy is damage to the nephrons of the kidney due to diabetes. When the kidney function is impaired, urea and creatinine levels in the blood become abnormally high. Therefore, urea concentration and creatinine clearance are used to assess kidney function.

Oxidative stress may be described as a disturbance in the oxidant/antioxidant balance within the cell, in which the oxidant prevails. It is a state in which a cell is experiencing alteration of cellular components, due to high exposure to free radicals and reactive oxygen species (ROS) beyond its antioxidant capacity. Although the reaction of ROS is essential for cellular functions, such as the utilization of chemical energy of nutrients for production of adenosine triphosphate (ATP), the generation of oxygen-free radicals is often formed through the stimuli of physical agents such as chemical, radiation, pollutants, and physiological processes which can disrupt mitochondrial function [2].

Hyperglycemia, oxidative stress, and complications of diabetes are closely linked together [3]. Increased levels of oxidative stress markers such as Oxidized Low Density Lipoprotein (oxLDL), Malondialdehyde (MDA), Advanced Oxidation Protein Products (AOPP), and Advanced Glycation End Products (AGEs) were reported in diabetes [4]. This is due to glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins [5].

Justicia Secunda Vahl (Family: *Acanthaceae*, Order*: Scrophulariales*, Sub-class: *Asteridae*, Class: Dicots) is a medicinal plant that originated from South America [6,7]. It is a creeping perennial plant with the ability to grow up to a height of about 1 to 1.5M. The plant is known as "blood root" in Barbados, "hounsiman" in Benin, meaning a plant that gives blood, and also referred to as "blood leaf" or "blood tonic" in some parts of Nigeria.

The leaf of *Justicia Secunda* is very potent in the treatment of diabetes and hypertension [8,9]. The leaf of the plant is purposely consumed as a decoction for improved packed cell volume (PCV) in certain parts of Nigeria, Congo, and Cote d'Ivoire [6]. It has been recognized among the species of *Justicia* endowed with antisickling [10], anti-inflammatory [11], antioxidant [12], antiviral and antimicrobial [13], superoxide anion radical scavenging [14,15], and wound healing potentials [16].

In this study, the focus is on determining the effect of *J. secunda* on oxidative stress, antioxidant activity, and renal function of diabetic Wistar rats. The study utilizes alloxan monohydrate (AMH), a chemical compound known to induce diabetes in animal models, to explore the effects of the extract in a controlled experimental setting.

2. 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. Autostrips), Chloroform (JHD Chemicals, Guangdong China), Hypodermic sterile 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), Latex Medical hand gloves (Supermax gloves, Selangor, Malaysia), Organ bottle, EDTA bottle, Standard pellet vital feed (grower), Lancet, 90(1) (Alpin Medical, England), UV-VIS 752N, and Ethanolic leaf extract of *Justicia Secunda*.

3. Methods

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

Experimental Design and Animal Grouping: Twenty-five (25) healthy Wistar rats weighing 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 a 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 fourteen (14) days. After the acclimatization period, the animals were injected with alloxan dissolved in sterile normal saline at a dose of 150mg/kgBW intraperitoneally. After 72 hours of the injection, the animals with fasting blood glucose (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 ethanolic leaf extract of *Justicia Secunda* was done as follows:

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

All administrations were done orally using oral cannula. At the end of the fourteen days treatment period with *Justicia 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. Blood samples obtained were put in well-labeled EDTA containers for further analysis.

- **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 air dried for three (3) weeks and then ground into powder using a sterile electric blender.

Two hundred and fifty grams (250 g) 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.

- **Determination of Oxidative stress level:** The blood samples collected were centrifuged, stored and the serum was used to assess the levels of oxidative stress marker, Malondialdehyde (MDA), which was determined using the colorimetric method as described by Gutteridge and Wilkins [17].
- **Determination of Antioxidant Activity:** The stored serum was used to assess the levels of the antioxidants: Superoxide Dismutase (SOD) and Catalase (CAT). SOD was assayed by colorimetric method as described by Misra and Fredovich (1972), while CAT activity was determined by colorimetric method described by Sinha [18].
- **Determination of Renal Function:** The serum collected was used to assess renal function by determining the levels of total urea concentration and creatinine clearance, which were analyzed spectrophotometrically with Randox assay kits.

3.1. 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 ($p \leq 0.05$) were considered to be statistically significant.

4. Results

Figure 1 showed that MDA level of Group N (Normal rats) was significantly lower when compared to Group Un D (Diabetes untreated). MDA level of Group Un D (Diabetes untreated) was significantly higher when compared to Group Met (Metformin). MDA level of Group Un D (Diabetes untreated) was significantly higher when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*). MDA level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 200mg + D (Diabetes + 200mg/kgBW ELE of *J. secunda*). MDA level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*). MDA level of Group 200mg + D (Diabetes + 200mg/kgBW ELE of *J. secunda*) was significantly higher when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*).

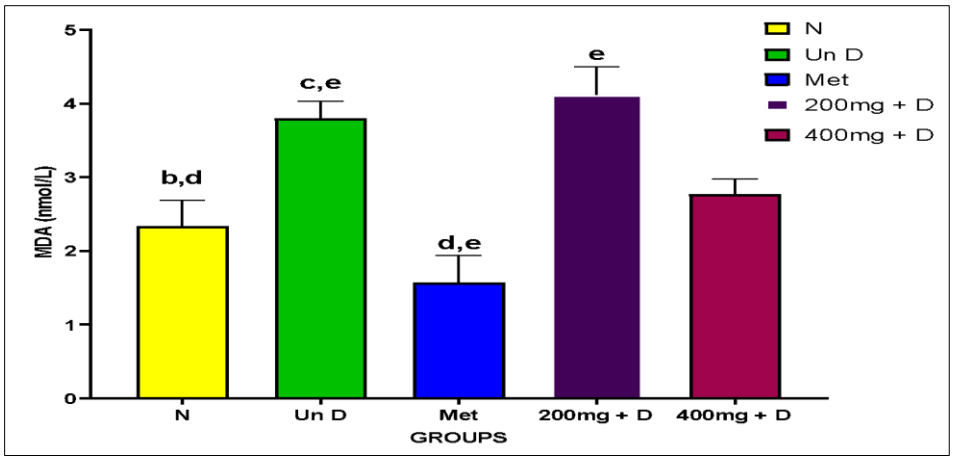

Figure 1 Effect of Ethanolic leaf extract of *Justicia Secunda* on MDA level in Alloxan-induced Diabetic Wistar Rats

Figure 2 revealed that SOD level of Group N (Normal rats) was significantly higher when compared to Group Un D (Diabetes untreated). SOD level of Group Un D (Diabetes untreated) was significantly higher when compared to Group Met (Diabetes + Metformin). SOD level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 200mg + D (Diabetes + 200mg/kgBW ELE of *J. secunda*). SOD level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*).

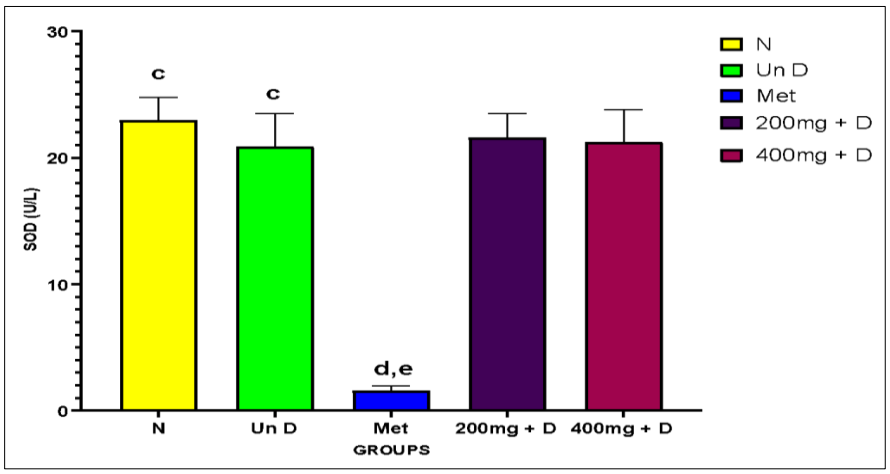

Figure 2 Effect of Ethanolic leaf extract of *Justicia Secunda* on SOD level in Alloxan-induced Diabetic Wistar Rats

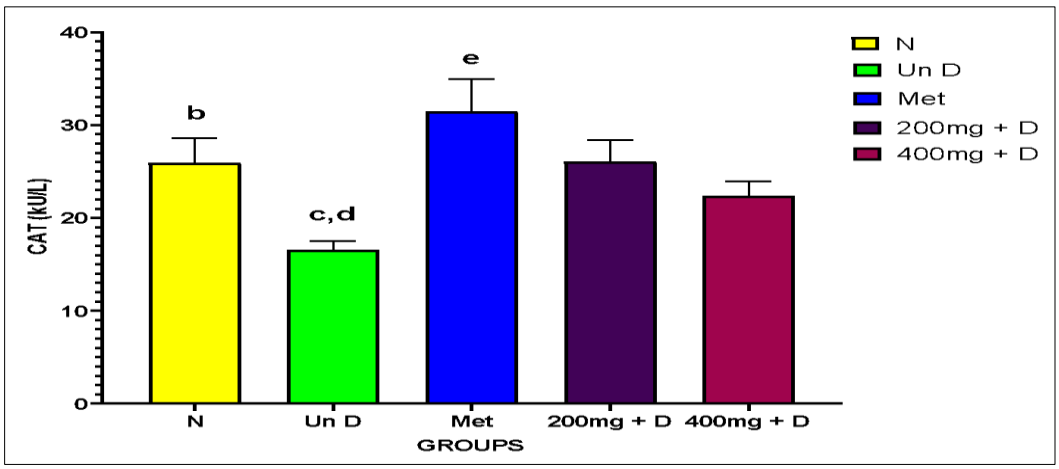

Figure 3 Effect of Ethanolic leaf extract of *Justicia Secunda* on CAT level in Alloxan-induced Diabetic Wistar Rats

Figure 3 showed that CAT level of Group N (Normal rats) was significantly higher when compared to Group Un D (Diabetes untreated). CAT level of Group Un D (Diabetes untreated) was significantly lower when compared to Group Met (Diabetes + Metformin). CAT level of Group Un D (Diabetes untreated) was significantly lower when compared to Group 200mg + D (200mg/kgBW ELE of *J. secunda*). CAT level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*).

Figure 4 revealed that Urea level of Group Un D (Diabetes untreated) was significantly higher when compared to group Met (Metformin). Urea level of Group Un D (Diabetes untreated) was significantly higher when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*). Urea level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 200mg + D (Diabetes + 200mg/kgBW ELE of *J. secunda*). Urea level of Group Met (Diabetes + Metformin) was significantly lower when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*). Urea level of Group 200mg + D (Diabetes + 200mg/kgBW of EJS) was significantly higher when compared to Group 400mg + D (Diabetes + 400mg/kgBW ELE of *J. secunda*).

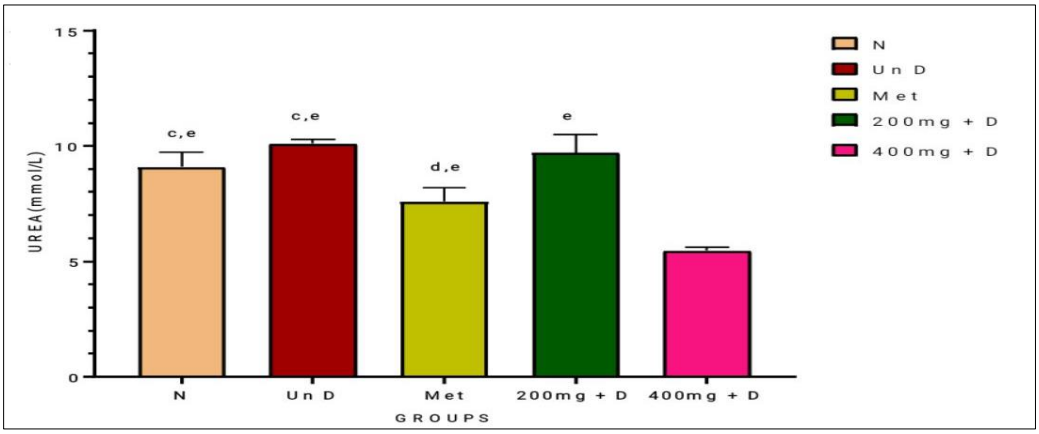

Figure 4 Effect of Ethanolic leaf extract of *Justicia Secunda* on Urea level in Alloxan-induced Diabetic Wistar Rats

Figure 5 revealed that Creatinine level of Group Un D (Diabetes untreated) was significantly higher when compared to Group Met (Metformin), and Creatinine level of Group Un D (Diabetes untreated) was significantly higher when compared to Group 400mg + D (Diabetes + 400mg/kgBW of ELE of *J. secunda*).

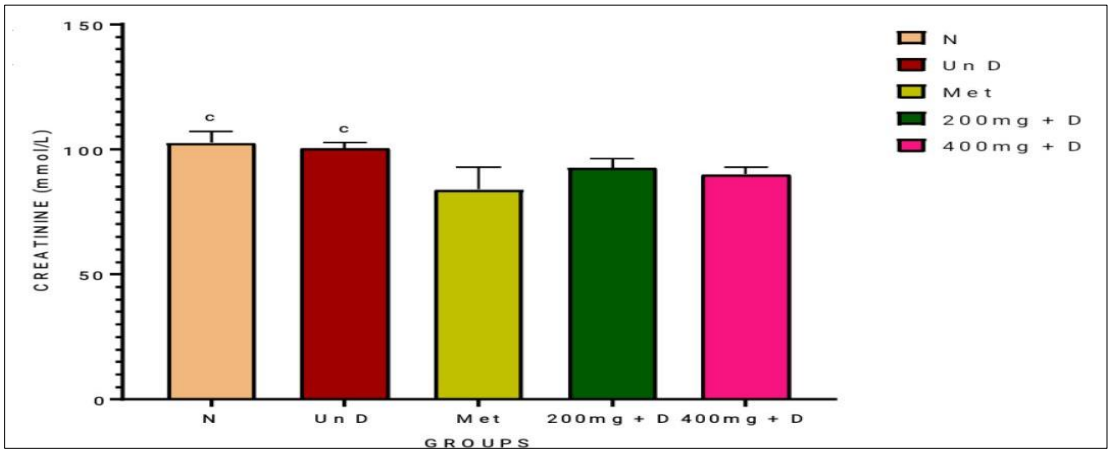

Figure 5 Effect of Ethanolic leaf extract of *Justicia Secunda* on Creatinine level in Alloxan-induced Diabetic Wistar Rats

5. Discussion

The term diabetes mellitus includes several metabolic disorders that, if left untreated, results in abnormally high glucose level in the blood. T1DM results from the inability of the pancreas to produce significant amounts of the hormone insulin, usually owing to the autoimmune destruction of the insulin-producing beta cells of the pancreas. T2DM, in contrast, is

now thought to result from autoimmune attacks on the pancreas and/or insulin resistance. The pancreas of a person with T2DM may be producing normal or even abnormally large amounts of insulin [19].

The increasing prevalence of T2DM worldwide has led to a concomitant rise in the prevalence of diabetes-related complications, including renal disease, and it is now widely accepted that oxygen species (OS) contribute to and plays a central role in the pathophysiology of T2DM, possibly via mediating the diversion of glycolytic intermediates [20].

Increased level of oxidative stress markers such as oxLDL [21], MDA [22], AOPP and AGEs [23] have been reported in diabetes. Hyperglycemia, oxidative stress and complications of diabetes are closely linked together [3].

Antioxidant defense is important in the removal of free radicals, providing the maximal protection of biological sites, such as thiol groups, which are part of active sites in some metabolizing enzymes. The most efficient enzymatic antioxidants involve SOD, CAT, and glutathione peroxidase. Nonenzymatic antioxidants involve thiol antioxidants (glutathione, thioredoxin, and lipolic acid), vitamin C, vitamin E, carotenoids, natural flavonoids, melatonin, and other compounds, such as selenium [24].

This study reveals that both doses of ethanolic leaf extract of *Justicia Secunda* used in this study (i.e. 200mg/kgBW and 400mg/kgBW) were able to significantly improve antioxidant levels of both SOD and CAT. The standard drug, Metformin was, however, found to be more effective in this regard. This agrees with the study findings of Ololade et al. [14], which reveal that *J. secunda* possesses antioxidant activity and reduces oxidative damage.

Also, the oxidative stress marker, MDA was reduced by 400mg/kgBW of the extract when compared to the untreated diabetic group (Un D). This also agrees with the study findings of [14]. 200mg/kgBW of ELE of *Justicia Secunda*, however, was not as effective in reducing oxidative damage as Metformin. High doses of *J. secunda* extract might be quite effective in ameliorating oxidative damage as the standard drug, Metformin. The mechanism of the in vitro antioxidant effect of the extract may be through scavenging of ROS-generating oxidases, and in vivo, may be via inhibition of the ROS-generating oxidases, enhancement of the endogenous antioxidant, and/or direct inhibition of the enzyme that catalyzes oxidation of cellular components [25,26]. The antioxidant effects of the extract may also be responsible for its anti-inflammatory and antinociceptive activities [26].

High levels of urea and creatinine in the blood may indicate impairment of kidney function and are therefore used as biomarkers for assessing the integrity of the kidneys.

Urea level in the untreated diabetes group was significantly higher when compared to the diabetic group treated with 400mg/kgBW ELE of *J. secunda*, and group treated with the standard drug, Metformin. There were also lower creatinine levels in groups treated with 200mg/kgBW and 400mg/kg BW ELE of *J. secunda,* but the decrease was not significant when compared to the effect of the standard drug, Metformin. Higher doses of ELE of *Justicia Secunda* may however, reveal significance. This study agrees with the study findings of Anyasor *et al.* [27], which revealed lower urea and creatinine levels in Wistar rats treated with ELE of *Justicia Secunda* when compared to the untreated groups.

6. Conclusion

This study postulates the role of oxidative stress and reactive oxygen species (ROS) in diabetes, and the effect of *Justicia Secunda* in managing the condition, as it reveals that ELE of *Justicia Secunda* was able to reduce oxidative stress by reducing the oxidative stress marker, MDA, and improving the antioxidant activities of SOD and CAT. This proves that it could be a good alternative to the standard drug, Metformin. The study also showed improvement in urea level and no change in creatinine level when the extract was administered to diabetic Wistar rats. However, higher doses may reveal significant changes in creatinine levels.

Compliance with ethical standards

Acknowledgement

The authors acknowledge the staff of the Department of Human Physiology, College of Health Sciences, Nnewi Campus, Nnamdi Azikiwe University, Nigeria.

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.

References

[1] Ismail K. Depression and Diabetes. Psychiatry. 2009, 8(6): 203-207.

[2] Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: impact on human health. Pharmacogn Rev. 2010, 4(8): 118- 26.

[3] Nowotny K, Jung T, Hohn A, Weber D, Grune T. Advanced Glycation End Products and Oxidative Stress in Type 2 Diabetes Mellitus. Biomolecules. 2015, 5(1): 194-222.

[4] Li W. Expression of cyclooxygenase-2 and inducible nitric oxide synthase correlates with tumor angiogenesis in endometrial carcinoma. Med. Oncol. 2005, 22(1): 63–70.

[5] So..zmen EY, So..zmen B, Delen Y, Onat T. Catalase/ superoxide dismutase (SOD) and catalase/paraoxonase (PON) ratios may implicate poor glycemic control. Arch Med Res. 2001, 32(4): 283-7.

[6] N'guessan K, Kouassi KH, Quattara D. Plants used to treat anaemia, in traditional medicine, by Abbey and Krobou populations, in the South of Cote- d'Ivoire. Journal of Applied Sciences Research. 2010, 6(8): 1291 – 2197.

[7] Kitadi JM, Lengbiye EM, Gbolo BZ, Inkoto CL, Muanyishay CL, Lufuluabo GL, Tshibangu DST, Tshilanda DD, Mbala BM, Ngbolu K, Mpiana P.T. *Justicia Secunda* Vahl species: Phytochemistry, Pharmacology and Future Directions: A mini-review. Discovery Phytomedicine. 2019, 6(4): 157-171.

[8] Theiler BA, Revoltella S, Zehl M, Dangl C, Caisa LOE, Konig J, Winkler J, Urban E, Glasl S. Secundarellone A, B, and C from the leaves of *Justicia Secunda* Vahl. Phytochem Lett. 2017, 10: cxxix–cxxxii.

[9] Carrington S, Cohall DH, Gossell-Williams M, Lindo JF. The antimicrobial screening of a Barbadian medicinal plant with indications for use in the treatment of diabetic wound infections. West Ind Med J. 2012, 61(9): 861–4.

[10] Kitadi JM, Lengbiye EM, Gbolo BZ, Inkoto CL, Muanyishay CL, Lufuluabo GL, Mazasa PP, Tshibangu DS, Kasali FM, Tshilanda DD, Ngbolua KTN, Mpiana PT. Ethnopharmacological survey and antisickling activity of plants used in the management of sickle cell disease in Kikwit city, DR Congo. Evid-Based Complement Altern Med. 2020, 1346493.

[11] Anyasor GN, Okanlawon, AA, Ogunbiyi B. Evaluation of anti-inflammatory activity of *Justicia Secunda* Vahl leaf extract using in vitro and in vivo inflammation models. Clinical Phytoscience. 2019, 5(49): 1-31.

[12] Goncalves-Filho D, De Souza D (2022). Detection of Synthetic Antioxidants: What Factors Affect the Efficiency in the Chromatographic Analysis and in the Electrochemical Analysis? Molecules. 2022, 27: 7137.

[13] Onochie AU, Oli AH, Oli, Obiajulu AN, Ezeigwe C, Nwaka AC, Okani CO, Okam PC, Ihekwereme PC, Okoyeh JN. The Pharmaco-biochemical Effects of Ethanol Extract of *Justicia Secunda* Vahl Leaves in Rattus Norvegicus. Journal of Experimental Pharmacology. 2020, 423-437. DOI: 10.2147/JEP.S267443.

[14] Ololade ZS, Anuoluwa IA, Salemcity AJ, Onifade OF. Secondary Metabolites, Anti-diabetic, Antioxidant, Anti-arthritic, and Antimicrobial Potential of *Justicia Secunda* for Health Benefits. Journal of Clinical Microbiology and Biochemical Technology. 2024, 10(1): 009-021.

[15] Osioma E, Hamilton-Amachree A. Comparative study on the phytochemical and in vitro antioxidant properties of methanolic leaf extract of *Justicia Secunda* Vahl. Nigerian Journal of Science and Environment. 2017, 15(1): 111-117.

[16] Kone WM, Koffi AG, Bomisso EL, Trabi FH. Ethnomedical study and iron content of some medicinal herbs used in traditional medicine in Cote D'ivoire for the treatment of anaemia. Afr J Tradit Complement Altern Med. 2012, 9(1): 81–7.

[17] Gutteridge JMC, Wilkins S. Copper-dependant Hydroxyl Radical Damage to Ascorbic Acid: Formulation of a Thiobabituric Acid-reactive Product. FEBS Letters. 1982, 137(2): 327-330.

[18] Sinha AK. Colorimetric Assay of Catalase. Analytical Biochemistry. 1972, 47(2): 389-394.

[19] Deshmukh SK, Verekar SA, Bhave SV. Endophytic Fungi: A Reservoir of Antibacterials. Frontiers in Microbiology. 2015, 5: 715

[20] McGrowder DM, Anderson-Jackson L, Crawford TV. In: Escher DA, editor. Biochemical evaluation of oxidative stress in type 1 diabetes. Type 1 diabetes. London: In Tech. 2013.

[21] Nakhjavani M, Esteghamati NA, Noworoozi S, Asgaranin F, Rashidi A, Khalizadeh O. Type 2 Diabetes Mellitus Duration: An Independent Predictor of Serum Malondialdehyde Levels. Singapore Medical Journal. 2010, 51(7): 582.

[22] Rashidi R, Rezaee R, Shakeri A, Hayes AW, Karimi G. A Review of the Protective Effects of Chlorogenic Acid against Different Chemicals. Journal of Food Biochemistry. 2022, 46(9): e14254.

[23] Esteghamati A, Larijani B, Aghajani MH, Ghaemi F, Kermanchi J, Shahrami A, Saadat M, Esfahani EN, Ganji M, Noshad S, Khajeh E, Ghajar A, Heidari B, Afarideh M, Mechanick JI, Ismail-Beigi F. Diabetes in Iran: Prospective Analysis from First Nationwide Diabetes Report of National Program for Prevention and Control of Diabetes (NPPCD-2016). Scientific Reports. 2017, 7: 13461.

[24] Rahman K. Studies on Free Radicals, Antioxidants, and Co-factors. Clinical Intervention in Aging. 2007, 2(2): 219-236.

[25] Leung HWC, Kuo CL, Yang WH, Lin CH, Lee HZ. Antioxidant enzymes activity involvement in luteolin-induced human lung squamous carcinoma CH27 cell apoptosis. European Journal of Pharmacology. 2006. 534(1-3): 12-18.

[26] Lin Y, Shi R, Wang X, Luteolin Shen H. A flavonoid with potentials for cancer prevention and therapy. Curr Cancer Drug Targets. 2008, 8(7): 634–46.

[27] Anyasor NG, Okanlawon AA, Ogunbiyi B. Evaluation of Anti-inflammatory effect of *Justicia Secunda* extract using in Vitro and in Vivo inflammation models. Clinical Phytoscience. 2020, 5:49.