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Dietary Management of *Vitex doniana* (Black Plum) Leaves in Alloxan-induced Diabetic Albino Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Diabetes is a major health concern and diabetes drugs have shown to produce adverse effect and therapeutically inefficacy overtime. This study investigated the effects of dietary effects of *Vitex doniana* leaves on fasting blood glucose levels in alloxan-induced diabetic rats; and explored dosage-response association between the concentrations of *V.doniana* leaves in the diet. The leaf was screened for the presence of phytochemicals using standard methodologies; alkaloids, terpernoids, flavonoids, phenol, tannins, saponins, reducing sugar, soluble sugar and non-reducing sugar, glycosides, and hydrogen cyanide, were found in the leaf. The result of the in-vivo study done for 21 days showed that there was significant difference (p < 0.05) between the blood glucose

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levels and body weights of rats in the treated group and the untreated group. The non-diabetic group had higher (p < 0.05) body weights than the untreated group, their blood sugar levels were lower (p < 0.05) than that of the induced untreated groups. The blood glucose levels of the induced untreated group were higher (p < 0.05) and their weights were reduced (p < 0.05) when compared with the blood sugar levels and weights of the treated groups. The blood sugar levels and weights of the rats that were treated with standard drug or *V.doniana* were similar (p < 0.05) to the blood sugar levels and weights of the standard group. The blood glucose levels of the rats treated with 20% *V.doniana* was higher (p < 0.05) than the blood glucose levels of rats treated with 40% *V.doniana*. The weights of the rats treated with 20% *V.doniana* was lower (p < 0.05) weight of rats treated with 40% *V.doniana*. The results suggest that *V.doniana* can be explored as a therapy for diabetic patients.

Keywords: Diabetes; phytochemicals; therapeutic effects; Vitex doniana; dosage-response.

1. INTRODUCTION

Diabetes is a prevalent and obstinate disease that is characterised by hyperglycaemia, a condition of high levels of blood glucose, and it is associated with morbidity as a result of vascular conditions [1]; the disease occurs when the pancreas produces insufficient insulin or when the body ineffectively uses the insulin it produces [2,3]. Globally, diabetes mellitus is said to have been on a significant increase between 2003 and 2019 [3], and Africa have been reported to have a great potential for the disease [2] In Nigeria, about 1.7 million people have been reported to be living with the disease, with diabetes Mellitus accounting for 2% of all mortality of all ages in the Country [1]. Over the years, studies on diabetes management have largely accepted alloxan-induced diabetes model as a practical model to test antidiabetic mediation [4,5]. Alloxan (2,4,5,6-tetraoxypyrimidine; 2.4.5.6pyrimidinetetrone), a glucose analogue is often used to induce diabetes in laboratory test animals [6] it is largely used to induce diabetes in experimental animals because it selectively destroys β -cells in the pancreatic islet cells by sequential changes leading to apoptosis [7]. This diabetogenic agent produces its effect by inhibiting insulin secretion through its ability to generate reactive oxygen species (ROS), resulting in the selective annihilation of β -cells [7]. Alloxan is used to study the pathophysiology of diabetes and evaluate potential therapeutic solutions [8]. Studies reviewing diabetes therapy suggest that conventional and synthetic therapeutic drugs are limited in terms of medication and these drugs have adverse side effects that could further lead to therapeutic inefficacy [9,10]. Thus, in recent years, there has been a growing interest in the therapeutic functionalities of folk medicinal plants [2]. A large number of traditional medicinal plants have been

identified to produce antidiabetic functions [2]. Plants offer a potential source of new biologically active compounds that may regulate glycemic control and improve metabolic parameters related to diabetes [11]. Vitex doniana (also known as Vitex doniana Sweet), a member of the Verbenaceae family is a deciduous mediumsized flowering tree that often grows as high as 15 metres to 20 metres in height in the forests of coastal tropical West Africa [12]. The plant is often referred to as Black Plum, and it is characterised by its "long-stalked leaves that are rounded at its apex with a glabrous leaflet and edible fruits" [12]. V. doniana is colossally available in the South-Eastern, North-Eastern, and Western parts of Nigeria [13,14]. The utility of this plant spreads across the three major ethnic groups of Nigeria, thus, it is locally identified as "Ucha Koro" in Igbo, "Dinya" in Hausa, and "Oori nla" in Yoruba. V. doniana is largely used in traditional medicine to treat diarrhoea, skin rashes, abdominal disorder, conjunctivitis, and lots more [12] it is further applied in the treatment of inflammatory disorders [15] mental illness, rheumatism, gastrointestinal disturbance, and urinary ailments [2]. Scientific studies although limited, have reported the antidiabetic potential of Vitex doniana on alloxan-induced diabetic albino wistar rats [16].

This study aimed to examine the effects of *Vitex doniana* leaves as a dietary management approach in alloxan-induced diabetic male albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Plant Materials

Mature leaves of Vitex doniana were harvested from Renaissance University's agricultural field in

Ugbawka, Nkanu West local government area of Enugu State. A plant taxonomist at the Department of Biology identified and confirmed them. The leaves were washed with running water to remove possible impurities, and they were air-dried at room temperature for two weeks. Afterwards, the dried leaves were pulverised into granules using a manual miller.

2.2 Qualitative Determination of Phytochemicals Contents of *Vitex doniana*

Phytochemicals were qualitatively determined by the methods of Harbone [17] Hikino et al. [18] and Edeoga et al. [19] as described in detail by Ogunjobi et al. [20].

2.3 Quantitative Determination of Phytochemicals

Phytochemical analysis was quantitatively determined by the methods of Harborne [17] Boham & Kocipai-Abyazan [20] Obadoni & Ochuko [21] and Amadi et al. [22] as described by Ogunjobi et al. [20].

2.4 Feed Formulation

Three experimental diets namely diet I (control feed), diet II (VDL 20%), and diet III (VDL 40%) were formulated using maize, wheat offal, groundnut cake, fish meal, bone meal, oil, limestone, vitamin premix, salt and powdered *Vitex doniana* leaf (VDL) according to the method described by Emeka and Obidoa [23] as shown in Table 1.

2.5 Nutritional Analysis of Feed

The proximate analysis of the feed was determined according to the method described by AOAC [24] ash content composition was determined according to the dry ash method described by AOAC (24) and James [25]. The crude fibre content was determined according to James [25]. The lipid content of the sample was using determined solvent extraction gravimetric method by Kirk and Sawyer [26]. The feed sample's protein content was examined using the Kjeldahl method described by Chang [27]. The carbohydrate was evaluated by difference. The evaluated percentages of crude fibre, crude fat, moisture, and crude protein were summed up and subtracted from 100%.

2.6 Animals

2.6.1 Experimental design

A total of 20 albino rats with body weight ranging from 130 to 200 g were used for this study. They were acclimatized for 1 week, and they were kept in metal cages and fed with rat chow and water *ad libitum*. The rats were used following NIH Guide for the use of laboratory animals. After the acclimatization period, the rats (in group 2, 3, 4 and 5) fasting blood glucose (FBG) was analysed using Accu answer kit then after were injected with alloxan monohydrate dissolved in distilled water in a dose of 0.5mg/ml intraperitoneally. After 48 hours of the injection, rats with fasting blood glucose (FBG) at or above 80 mg/dL were considered diabetic.

2.6.2 Grouping of animals

The animals were randomly assigned to 5 groups of 4 rats each, and treated as follows:

Group 1: The normal group were given the diet I and water. Group 2: The negative control group were given the diet I and water, were not treated. Group 3: The positive control group were given diet I and water, were treated with 10µL 5mg/kg metformin and 500mg/kg glibenclamide daily, orally. Group 4: This group were given a diet II (20% of *Vitex doniana* leaf) and water. Group 5: This group were given diet III (40% of *Vitex doniana* leaf) and water. The feed and treatment were administered daily for 21 days.

2.6.3 Determination of blood sugar levels

The blood sugar levels of the rats were determined before the induction and 48 hours after the induction of diabetes by drawing blood from the tail-tip of the animals. Their glucose level was tested using a Finetest glucometer. The blood sugar level was checked weekly within the 21 days.

2.6.4 Determination of body weight

The rats were weighed at the commencement of the experiment and the initial weight was recorded. The final weights of the rats were recorded at the end of the experiment and overall weight gain was obtained by subtracting the initial weight from the final weight. Daily weight gain was determined by dividing the overall weight gain by the total number of days of the experiment.

Feedstuff	Diet I (Control)	Diet II {V.doniana (20 %)}	Diet III {V.doniana {(40 %)}
Maize	50	40	30
Wheat Offal	26	16	6
Groundnut cake	9.6	9.6	9.6
Fish meal	6	6	6
V.doniana 20%	-	20	-
V.doniana 40%	-	-	40
Bone meal	2.0	2.0	2.0
Oil	4.0	4.0	4.0
Limestone	2.0	2.0	2.0
Salt	0.2	0.2	0.2
Premix Vitamins	0.2	0.2	0.2
Total (Approx.)	100g	100g	100g

Table 1. Formulation of diets for different groups of rats (g/%)

2.7 Statistical Analysis

Statistical Analysis All the parameters studied were analysed statistically by comparison of mean tests using SPSS version 21. Data were expressed as Mean \pm SD of three replicates and differences were considered statistically significant at p < 0.05.

3. RESULTS

The result of the proximate analysis of the feeds showed high volumes of carbohydrate, protein, and moisture in diet I, diet II, and diet III. However, the fat content of diet III and diet II is higher than the fat content in diet I. The result shows that the ash content of the three diets slightly differs.

The result of the blood sugar test done within the experiment duration showed that there was significant increase in the blood sugar of groups 2, 3, 4, and 5. However, the treated groups (groups 3, 4, and 5) had reduced blood sugar levels which were only slightly different from the positive control group (1) at the final week. Group 2 still had a significantly elevated blood glucose level at the end of the final week.

3.1 Result of the Quantitative and Qualitative Analysis of Phytochemicals in *Vitex doniana*

The result of the quantitative and qualitative analysis of phytochemicals in *Vitex doniana* leaf sample showed high presence and high quantities of tannins, soluble sugar, flavonoids, terpenoids, phenol, and alkaloids in *V. doniana*, as against the low presence and little quantities of hydrogen cyanide, saponins, and glycosides; reducing sugar, non-reducing sugar, and steroids were moderately present.

3.2 Result of the Body Weight of the Rats

The result of the body weight showed that there was a significant weight loss post alloxan induction. There was weight reduction in the untreated group (group 2), the weight of the treated groups (group 3, 4, and 5) increased across 21 days. Overall, the weights of the treated groups were only slightly different from the weight of the positive control group 1 at the final week.

Table 2. Result of the com	parative proximate	composition analy	ysis of diets fed to the rats

Parameters	Diet I	Diet II	Diet III
Moisture (%)	10.88± 0.16 ^a	11.07± 1.01 ^b	11.86± 0.25 ^c
Crude fibre (%)	5.01 ± 0.14^{a}	8.11± 0.83 ^b	9.77± 0.43°
Crude (fat %)	12.05± 0.81ª	13.00± 0.71 ^b	13.03± 0.83ª
Ash (%)	8.73± 0.73 ^a	9.07± 0.64 ^b	9.81± 0.22 ^c
Protein (%)	23.72± 0.55 ^a	25.06± 0.32 ^b	26.03± 0.63 ^b
Carbohydrate (%)	39.61± 0.49°	33.69± 0.72 ^b	29.50 ± 0.47^{a}
Metabolizable energy (Kcal/Kg)	359.77±11.47ª	352.00±10.55 ^b	339.29±11.87°

Phytochemicals	Qualitative	Quantitative
Tannins	+++	17.97±0.21 (mg/100g)
Hydrogen cyanide (HCN)	+	0.71±0.15 (mg/100g)
Soluble sugar	+++	22.18±0.20 (mg/100g)
Reducing sugar	++	8.71±0.48(mg/100g)
Non-reducing sugar	++	11.87±0.52 (mg/100g)
Flavonoids	+++	32.22±0.24 (mg/100g)
Saponins	+	0.95±0.10 (mg/g)
Steroids	++	3.50±0.28 (mg/g)
Terpenoids	+++	49.24±0.32(mg/100g)
Phenol	+++	38.48±0.21(mg/100g)
Alkaloids	+++	34.88±0.29(mg/100g)
Glycosides	+	0.84±0.17 (mg/100g)

Table 3. Results of the quantitative and qualitative analysis of phytochemicals in Vitex doniana

3.3 Result of the Blood Sugar Levels of the Rats

The result of the blood sugar test done within the experiment duration showed that there was significant increase in the blood sugar of groups 2, 3, 4, and 5. However, the treated groups (groups 3, 4, and 5) had reduced blood sugar levels which were only slightly different from the positive control group (1) at the final week. Group 2 still had a significantly elevated blood sugar level at the end of the final week.

4. DISCUSSION

The result of this study showed that the moisture content of diets (diet II and III) containing Vitex doniana is slightly higher than the moisture content of diet I (Table 2). The moisture content of diet I, diet II (20% V. doniana), and diet III (40% V. doniana) were 10.88 ± 0.16, 11.07 ± 1.01, and 11.86 ± 0.25 respectively. The results of diet II and diet III were similar to the analysis of Abdullahi et al. [28] however, they were lower than the result obtained by Ifeanacho & Ogunwa [29]. The disparity in the moisture content of the diets is due to concentration of moisture content in the feedstuff used to prepare the diets; The significance of moisture content in food is relative to the shelf life of the food. Foods with high moisture content (above 10 %) have shorter shelf life and they are prone to microbial activity and deterioration. Protein is necessary for rebuilding tissues, hormones, blood cell concentration, and energy balance. The protein content of the diets is relatively high; however, the protein content of diet II (25.06 ± 0.32) and diet III (26.03± 0.63) were higher than diet I (23.72± 0.55). The protein contents of the diets were higher than the results reported by Abdullahi et al. [28]. However, they were similar to the results obtained by Ifeanacho

& Ogunwa (29). The fibre content of diet I (5.01 ± 0.14) was lower than diet II (8.11± 0.83) and diet III (9.77 ± 0.43). The protein contents of diet I was lower than that observed by Ifeanacho & Ogunwa [29] and Abdullahi et al. [28]. The protein contents of diet II and III were relatively similar to the report of Ifeanacho & Ogunwa [29] but they were lower than the results reported by Abdullahi et al. [28]. Dietary fat is a good source of energy for humans. There was no much difference in the crude fat content of the diets used for this study: howbeit, diet I (12.05 ± 0.81) was lower than diet II (13.00± 0.71) and diet III (13.03± 0.83). The results of the fat content (across the diets) obtained in this study was relatively similar to the those reported by Abdullahi et al. [28]. The fat content reported by Ifeanacho & Ogunwa [29] were far lower than that obtained in this study. The vast variance between the crude fat content of the diets in the study can be traced to the addition of vegetable oil in the diets. The ash contents of the diets used in this study differ slightly: diet I contained 8.73± 0.73, diet II contained 9.07± 0.64, and diet III contained 9.81± 0.22; but they were relatively similar to the data reported by Ifeanacho & Ogunwa [29]. The ash content of feed reported by Abdullahi et al. [28] was higher than that reported in this study. Carbohydrate is essential for energy and amino acid metabolism. The carbohydrate content of diet I (39.61 ± 0.49) was significantly higher than the carbohydrate content of diet II (33.69 ± 0.72) and diet III (29.50 ± 0.47), and diet II was higher than diet III. This variance is could be due to the differences in the volume of maize and wheat offal that was used in the feed formulation. The carbohydrate content reported by Ifeanacho & Ogunwa [29]. and Abdullahi et al. [28] was higher than data reported in this study. The energy level of the diets was 359.77±11.47 (diet I), 352.00±10.55

(diet II), and 339.29 ± 11.87 (diet III). Diet I supplied more energy compared to the other feed, while the 40% had the lowest energy supply.

Phytochemicals are naturally occurring bioactive compounds in plants. The therapeutic effects of medicinal plants have been associated to their phytochemical constituents. Several studies have reported phytochemicals to reduce and prevent ailments chronic in animal models. Phytochemical analysis is employed to identify the qualitative and quantitative presence of phytochemicals in plants. The results of the quantitative and qualitative analysis of phytochemicals in Vitex doniana leaves revealed the presence of phytochemicals like alkaloids, terpernoids, phenols, and tannins, reducing sugars, glycosides, hydrogen cyanide, and saponins. Alkaloids, terpernoids, phenols, and tannins were highly present in V.doniana leaf (Table 3). Sugars (reducing and non-reducing sugars) were very much present, confirming the high carbohydrate content of the leaf. Hydrogen cyanide, saponins, and glycosides were present in low quantities. The result of the phytochemical analysis done in this study compares well with the result that was reported by Odika et al. [30]. who studied the bioactive profiling of Vitex doniana leaf and its effect on rat erythrocyte membrane stabilization activity. Alkaloids. terpernoids. flavonoids. phenol, tannins. saponins, reducing sugar, soluble sugar and non-reducing sugar, glycosides, and hydrogen 34.88±0.29mg, cyanide were present in 49.24±0.32mg, 32.22±0.24mg, 38.48±0.21mg, 0.95±0.10ma. 8.71±0.48ma. 17.97±0.21ma. 22.18±0.20mg, 11.87±0.52 mg, 0.84±0.17mg, 0.71±0.15mg per 100g respectively. Alkaloids are instrumental in the treatment of tumours and diarrhoea [30]. Hydrogen cyanide is known to influence respiration and exude toxic effects [31]. Terpenoids have been reported to manifest antiinflammatory and antidiabetic activity [32]. thus, the abundance of terpernoids in Vitex doniana may constitute the antidiabetic potential of the leaf. Phenols and flavonoids are phenolic acids, and they have been identified to be effective against high fat induced hyperlipedamia and oxidative stress by regulating insulin secretion [30]. Reducing sugars are digestible and can increasingly affect blood sugar. The presence of reducing sugars may signify the hyperglycemic effect of Vitex doniana on blood sugar levels. Saponins are reported to protect against pathogens, and exert antimicrobial, antiinflammatory, and antiulcer properties [32]. On

the other hand, glycosides have been identified to produce antiviral, antibacterial and analgesic effects [33]. The presence of saponins and glycosides would mean that *Vitex doniana* may be exploited in antibacterial therapy.

The result of the body weights of the animals that were used for this study showed that the animals reduced weight 48 hours after the induction of alloxan monohydrate (Table 4). The table shows that there was a slight improvement in the weight of the animals in the groups that were treated with normal drug and Vitex doniana diets in the first week. The results showed that the rats gained increased weight over the next 14 days when compared with the standard group. The groups that were treated with Vitex doniana diets significantly had more weight than the group treated with metformin and glibenclamide. The untreated group had steady body weight decline post alloxan induction which can be due to the activity of alloxan and diabetes. The increased body weight of the rats treated with normal drug and V.doniana diet is due to the ameliorating activity of the normal drugs (metformin and glibenclamide) and Vitex doniana diets. Corcoran Jacobs [34] reported that & metformin moderately reduces weight while exerting blood sugar reducing effect; but glibenclamide has been reported to increase weight [35]. The complimentary use of both drugs may cause the slight difference in the weights of the rats when compared with the standard and Vitex doniana groups. The weight gained by the groups treated with Vitex doniana is due to the reduction of their blood sugar levels. The group treated with 40% Vitex doniana diet lost more weight than the group treated with 20% Vitex doniana, due to the increased dosage of the nutrient in 40% Vitex doniana. The body weight results obtained in this study agree with the work of Ani et al. [36] who studied the inhibitory potential and antidiabetic activity of extracts of Vitex doniana leaf.

Blood sugar is a biomarker of diabetes, evaluating its levels in the management of diabetes is essential to determine the effects of therapy understudy. The results of the blood glucose levels of this study showed that the blood glucose of the animal models increased 48 hours after the induction of alloxan monohydrate (Table 5). The results of the first week showed slight reduction of the blood sugar levels of the animals treated with standard drug and *Vitex doniana* diets, daily. The sugar levels of the untreated consistently increased over the next 14 days, while the sugar levels of the treated groups

Days	Group 1	Group 2	Group 3	Group 4	Group 5
Initial	189.77±12.62 ^a	200.77±5.36 ^b	213.47±8.12°	222.05±2.75 ^d	235.60±0.20 ^e
Post	195.07±15.55 ^a	195.60±4.32 ^a	205.93±8.64 ^b	208.25±19.55 ^b	228.35±14.55°
Induction					
Week 1	194.63±10.09 ^b	189.50±4.58 ^a	210.93±8.92 [°]	218.65±28.15 ^d	223.90±10.00 ^e
Week 2	207.73±25.91 ^b	184.27±5.95 ^a	218.43±16.25℃	225.55±6.59 ^d	224.15±12.55 ^d
Week 3	215.87±24.99 ^b	186.47±6.74 ^a	226.57±13.53℃	234.40±3.24°	230.85±18.95 ^d

Table 4. Result of the body weight of the rats

Values are represented as mean \pm standard deviation. The mean differences for the values with the same superscript within the group have no statistical difference at $p \ge 0.05$.

Days	Group 1	Group 2	Group 3	Group 4	Group 5
Initial	42.00±3.60°	40.33±5.56 ^a	47.00 ± 4.55^{d}	67.50 ± 7.50 ^e	41.00±3.00 ^b
Post	56.00±1.00 ^a	115.00±17.66 ^d	100.33±18.37℃	85.50±10.50 ^b	117.00±26.00e
induction					
Week 1	55.00±2.10 ^b	123.00±10.23 ^e	74.33±14.88 ^d	68.00±10.00 ^c	53.50±15.50 ^a
Week 2	57.00±2.50ª	128.33±23.61 ^e	68.67±19.60 ^d	63.50±2.50 ^b	64.00±11.00 ^c
Week 3	61.33±4.71 ^b	124.00±12.33 ^d	51.67±11.15 ^a	63.50±2.50°	61.50±10.50 ^{bc}

Values are represented as mean \pm standard deviation. The mean differences for the values with the same superscript within the groups have no statistical difference at $p \ge 0.05$.

reduced over the same time. The blood sugar levels of the groups treated with V.doniana diet showed to be similar with the non-diabetic group, while the sugar level of the metformin and glibenclamide group was lower than the nondiabetic group and V.doniana groups at the end of the final week. This variance can be traced to the pharmacological action of metformin and glibenclamide: metformin reduces blood sugar by decreasing glucose production in the liver, increasing insulin sensitivity, reducing insulin levels, and decreasing intestinal absorption [34] while glibenclamide reduce blood sugar levels by increasing insulin secretion from beta cells in the pancreas by binding to the SUR1 receptors of in the membrane of the beta cells of ATP dependent channels; blocking the channels and catalysing the release of insulin from the depolarised cell [35]. The glucose level of the 40% Vitex doniana group was slightly lower than those treated with 20% Vitex doniana. The difference between the groups treated with Vitex doniana can be traced to the increased dosage of Vitex doniana in their diet. Whereas the reduced difference between the metformin and glibenclamide group, and the non-diabetic group and Vitex doniana group can be associated to the mechanism of action of metformin and glibenclamide in the animals. The results of this study were similar to the reports of Ani et al. [36] and Akosu et al. [37]. The antidiabetic activity of Vitex doniana can be traced to their ability to inhibit the activity of two carbohydrate

metabolising enzymes– α -amylase and α -glucosidase [36,37]. The reduced blood sugar levels in the groups treated with *Vitex doniana* diet may also be linked to the high fibre content of *Vitex doniana* leaf which slows down the metabolism of carbohydrate in the body.

5. CONCLUSION

The evaluation of the therapeutic effects of whole Vitex doniana leaves in diets was necessary to ascertain the antidiabetic activity of the leaves and their role in nutrition. The results of the analysis showed that Vitex doniana leaves were highly nutritious and contain phytochemicals of pharmacological interest; the results also showed that Vitex doniana diets reduced blood glucose levels and manifested antidiabetic activity in diabetic rats. The nutrient composition of the plant leaves showed that it can be used like other edible vegetables in our homes. The phytochemical composition showed that Vitex doniana can be developed for therapy against other diseases alongside diabetes.

6. RECOMMENDATION

We recommend that global and national agencies advocate for the consumption of *Vitex doniana* leaves in family homes. *Vitex doniana* can also be explored as supplements; and special meals for diabetes patients.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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