



Evaluation of Selected Novel Delicacies of Wild Plants Using Wistar Rats: An Insight into Nutritional Quality

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Abstract

The quest to improving the nutritional quality of a growing population is critical. Nutritional quality is lacking in terms of which vegetable will yield the desired nutrient. This research investigates the nutritional quality of some wild edible vegetables and their effect on rats. Two different delicacies were prepared with two novel vegetables - *Adenia cissampeloides* (ACD) and *Arthropteris Palisoti* (APD) plants. The mineral and vitamin profiles in these vegetables were determined using standard methods. Twenty four weanling rats with weight ranging from - 43.99 to 81.49 g, were randomly designated into four groups (n = 6). Two groups of the experimental rats were fed with the formulated experimental diets, while the other two groups were fed with protein-free (casein) and basal diets. Carbohydrate, protein, vitamins C and E were significant at $p < 0.05$ in the two wild vegetables; the mineral composition showed significance at $p < 0.05$ for delicacies with low Na^+ content while Ca^{2+} concentration was significantly high in ACD and APD. Mg^{2+} was high in ACD while Phosphorus concentration was high in APD. The ACD-fed rats had a higher value (2.37 ± 0.01 %) compared to APD (2.18 ± 0.01). The reference group consumed more food (97.06 ± 14.70 g) followed by the basal group (88.98 ± 10.61), ACD (43.89 ± 14.34), and APD (42.02 ± 7.98), respectively. There was no significant differences ($p > 0.05$) observed in the body weight changes, protein efficiency ratio, net protein utilization, net protein retention, true digestibility, fecal and carcass protein levels in all the groups. Findings suggest that nutrients in these vegetables are of good quality to benefit the user hence it is recommended in routine diet preparations.



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Introduction

Wild edible plants are botanical species that are neither domesticated nor cultivated but exist in their natural habitats.¹ Distribution of plant species is influenced by drought-resistant ability, reproduction, and regeneration.² Wild plants are tolerant, resilient and adaptive to adverse environmental conditions; they can thrive in any geographical zone.³ They are abundant varieties of edible plants found in the wild.⁴ According to Food and Agricultural Organization (FAO) estimate, a minimum of one billion individuals use wild edible plants (WEP) in their diets.¹ In Swaziland, WEP constitutes a greater portion of diets than cultivars while over 300 species of wild leafy vegetables and fruits are consumed in Ghana.⁵ In Nigeria, about 42 wild edible plants belong to 27 scientific families identified by the Tiv people of Benue State-Nigeria. *Amaranthus tricolor*.⁶

Wild plants cooked as vegetables include *Ficus lacor*, *Smilax aspera*, *Hydnium repandum*, *Ficus hispida*, *Acacia rugata*, *Capparis spinosa*, *Bambusa nepalensis*, *Dillenia pentagyna*, *Urtica dioica*, *Remusatia vivipara*, etc. Another WEP is consumed raw as fruits e.g. *Morus nigra*, *Cissus adnata*, *Zizyphus mauritiana*, *Ficus racemosa*, *Piper longum*, *Ficus auriculata*, *Coccinia grandis*, *Antidesma acidum*, *Mangifera indica*, *Rhus javanica*. In addition, certain WEP is used as spices (*Murraya koenigii*, *Cleome viscosa*, and *Cinnamomum Tamala*) in various traditional delicacies. In Bardiya district (Nepal), fruits gotten from *Acacia rugata* are used as a detergent.⁷ This increasing fascination in WEP is chiefly due to their high content of macronutrients and micronutrients.⁸ An example is the edible wild *Zygophyllum album* which is reported to contain 25.20 mg, 20.83 mg, 8.67 mg and 3.52 mg of potassium, calcium, magnesium, and phosphorus, respectively per gram of dry weight in its shoot/leaves [9]. Therefore, identifying and incorporating such veritable WEP in human diets will substantially address the nutritional challenges of vulnerable populations and cushion the effects of food paucity during critical/desperate times. The WEP is laden with pharmaco-active compounds with numerous medicinal applications such as a diuretic, anti-inflammatory and aphrodisiac.¹⁰ This explains why they are tagged "functional foods".

Functional foods (otherwise called nutraceuticals or food supplements) are capable of providing biochemical substances for nutritional and therapeutic purposes.¹¹ It has been established that regular consumption of edible fruits, leaves and other parts of wild plants lowers the risk of cancer, diabetes mellitus, neurodegenerative disorders and cardiovascular diseases.¹² Since antiquity, many people have been using wild plants (commonly referred to as herbs) for the management/treatments of various diseases.¹³ In fact, available pharmaceutical drugs are indirect or direct products of wild plants. Evidently, compared to synthetic drugs the side effects of such herbal plants are minimal owing to the biodegradable nature of some of their anti-nutrients which can be effectively metabolized by the human system.¹⁴ Information on the nutritional benefits of lesser known vegetables are quite few in literature; this study was therefore designed to evaluate the nutritional quality of *Adenia cissampeloides* (ACD) and *Arthropteris Palisoti* (APD) based- diets.

Materials and Methods

Collection of Vegetables and Identification

The wild plants were selectively collected from the central senatorial district of Cross River State, Nigeria. There are called different names such as *Adenia cissampeloides* "Igwu" by the people of Yala-Nkum in Ikom and *Arthropteris Palisoti* "Ikpalad" (Ekori, Yakurr LGA). They were profiled and authenticated by a botanist, Dr. S. Udo of the Department of Botany, Cross River University of Technology (CRUTECH), Calabar.

Processing Delicacies

The vegetables were washed cleaned under running tap water and used to process the delicacies based on the local recipes of the study area - *Adenia cissampeloides* delicacy (ACD), and *Arthropteris Palisoti* delicacy (APD). Besides the wild plants, other food ingredients which were used for the preparation of the delicacies were bought from Watt market in Calabar, Cross River State, Nigeria.

Thereafter, the delicacies were thoroughly mixed and oven dried at 50°C for 24 hours. The dried samples were ground into powder using mortar and pestle

after which it was stored in airtight containers and kept in a refrigerator (4 °C) before being used for subsequent analyses.

Estimation of Vitamin E

One gram of each goodies sample was put in a clean test-tube, macerated for 10 min with

n-hexane solution (20 ml) and then centrifuged at 3000 rpm (using a Camlab desktop centrifuge, Cambridge) for another 10 min. Later, 3.0 ml of the supernatant (filtrate) was aspirated in a test-tube, and evaporated to dryness using a water bath (Labotec water bath, Durban).¹⁵

Table 1: The composition of the items purchased for the experiment

Components	Basal feed	Reference feed	Trial goodie 1 (ACD)	Trial goodie 2 (APD)
Corn-starch	840	720	354	166
Casein	-	120	-	-
Trial material	-	-	486	674
Sucrose	120	120	120	120
Glucose	60	60	60	60
Soy oil	60	60	60	60
Cellulose	60	60	60	60
Vitamin mix	12	12	12	12
Mineral mix	48	48	48	48
Total	1200	1200	1200	1200

The cornstarch was bought from marina market, Calabar while the components of mineral and vitamin mix were purchased from a chemical shop in Calabar.

Trial delicacy 1: *Adenia cissampeloides* goodie (ACD)

Trial delicacy 2: *Arthropteris Palisoti* goodie (APD)

Basal diet: Protein-free feed

Reference diet: Casein-based feed

Feeding of Experimental Animals

Twenty four Wistar rats of body weight 43.99 - 81.49 g were purchased from the animal house of the Department of Biochemistry, University of Calabar. The rats were weighed (using Vaman electronic balance, Mumbai) and randomly distributed based on average body weight into four (4) groups (n = 6). The rats in each experimental cluster were housed in ventilated metabolic cages, acclimatized for three days with access to the six experimental diets for 10 days. Hygienic conditions were ensured

and maintained on a 12:12 hr light/dark cycle at temperature 25 - 27°C. The animals' feeding was designed as follows:

Group A. received *Adenia cissampeloides* delicacy (ACD),

Group B - *Arthropteris Palisoti* delicacy (APD),

Group C - Basal (protein-free) diet and group

D - Reference (casein) diet.

The delicacies and diets (20 g) were made available to each rat daily while clean water was given *ad libitum*. The initial body weights of the animals were taken at the beginning of the experiment, subsequent measurements was done every other day and after the treatment interval of (10 days) using an analytical scale. The spilled/leftover foods and the fecal remains were removed daily for measurements and analysis. The food intake was calculated by subtracting the weight of the spilled and leftover delicacies from the total amount of delicacies administered. At the end of the treatment, the rats were made to fast

(although they had free access to water) 24 hours before they were sacrificed. The sacrifice was done carefully on a filter paper to avoid spillage of blood. Both rats and filter paper were dried in an oven at 105°C for 48 hr. They were then crushed, blended and stored properly for analyses. Furthermore, the collected feces were also dried in the oven, blended and preserved adequately. The fecal matter and the rat carcasses were both kept in the refrigerator for analyses.

Analysis of Physiological Parameters

Diet, carcass, and feces were analyzed for nitrogen (N) content according to the method described by.¹⁶ All parameters were determined following the method described by.¹⁷ Protein efficiency ratio (PER) was extrapolated by relating the weight gained to the amount of protein eaten-up, see equation 1.

$$\text{PER} = \text{wt gain (gm)} / \text{protine intake gm} \quad \dots(1)$$

The net protein utilization (NPU) was estimated by finding the in carcass nitrogen difference between rats fed with the test diets and those fed with the protein-free diet, see equation 2 or 3.

$$\text{NPU} = \frac{\text{carcass N of test group} - \text{carcass N of the basal group}}{\text{intake of test group}} \quad \dots(2)$$

or

$$\text{NPU} = \frac{\text{N retained} \times 100}{\text{N intake}} \quad \dots(3)$$

The net protein Ratio (NPR) was calculated estimating the body weight differences between the test group and the basal (protein free) group, using equation 4.

$$\text{PR} = \frac{\text{weight gain on test diet} - \text{weight loss on basal diet}}{\text{protein ingested by the test group}} \quad \dots(4)$$

The true digestibility (TD) was determined based on the nitrogen that was eaten-up and fecal nitrogen using equation 5 or 6.

$$\text{TD} = \frac{\text{intake (fecal N on test diet} - \text{fecal N on basal diet)} \times 100}{\text{in take}} \quad \dots(5)$$

The biological value (BV) was derived, using equation 6 or 7.

$$\text{BV} = \frac{\text{NPU} \times 100}{\text{TD}} \quad \dots(6)$$

$$\text{Or BV} = \frac{\text{N retained} \times 100}{\text{N absorbed}} \quad \dots(7)$$

Or

Mineral elements were determined following the methods of.¹⁵ Calcium and Magnesium were determined by the method of.¹⁸ Phosphorus.¹⁹ and Sodium.²⁰

Statistical Analysis

Statistical package for service in science (version 20.0) was used to analyze the data. Results are presented as mean \pm standard deviation. ANOVA and significant differences was accepted at $p < 0.05$ degree of confidence, followed by the least square difference and post-hoc test.

Result and Discussion

Investigation of the nutrition quality of some novel wild plants was carried out, their effects on the rat's system were monitored. Some reactions involved chelating of anti-nutrients to minerals such as calcium, magnesium, sodium, and phosphorus. These in parts explain the differences in the mineral levels of the delicacies. It is plausible to say that some minerals may have leached into the cooking fluid; this agreed with²¹ who states minerals are not destroyed by heat that during cooking, but leached into the liquid medium. Minerals (otherwise called micronutrients) though constitute only 4.0-6.0 percent of the human body are essential to human health if consumed. The body requires micronutrients to function. Calcium plays a participatory role in some biochemical processes such as clotting, activation of enzymes, neuromuscular stimulation, formation and development of bone and teeth.²²

Intracellularly, the most abundant divalent cation is magnesium. It helps with the sustainance of cardiovascular activities; function as essential cofactor of some enzymes.²³ Sodium and phosphorus are well implicated in the transportation of

bio-molecules, generation of chemical energy and maintenance of homeostasis.²⁴ Similarly, both the cooking and leaching processes may be responsible for the differences in vitamins A, C, and E concentration in different delicacies, as previously reported,²⁵ that cooking and leaching induce changes in the vitamin composition of vegetables. Besides its numerous functions, vitamin A (retinol) is fundamental to clear eyesight.¹⁵ Vitamins C (ascorbic acid) and E (tocopherol) are pertinent antioxidants that facilitate the scavenging of free radicals or oxidants. Thereby, preventing diseases and promoting good health.²⁶ The differences in the nutrients and anti-nutrients composition of the

various delicacies were attributed to plant genetics and maturity stage of the vegetables.²⁷

Vitamin Content

Vitamin A ($\mu\text{g}/100\text{ g}$) expressed detectable differences among the delicacies at $p < 0.05$ (Table 1). A significant difference across the samples was observed in the case of vitamin C content ($\text{mg}/100\text{ g}$) whereby *Arthropteris Palisoti* (APD) and *Ficus glumosa* (FGD) had both the highest and lowest concentration ($p < 0.05$). Table 2 showed that *Adenia cissampeloides* delicacy (ACD) had the highest content ($\text{mg}/100\text{ g}$) of vitamin E.

Table 2: Selected vitamins compositions of delicacies

Delicacies	Vitamin A ($\mu\text{g}/100\text{ g}$)	Vitamin C ($\text{mg}/100\text{ g}$)	Vitamin E ($\mu\text{g}/100\text{ g}$)
ACD	86.50 \pm 0.36	70.93 \pm 2.71	8.10 \pm 0.20
APD	77.59 \pm 0.43	76.70 \pm 0.78	7.33 \pm 0.21

The trial groups with the same superscripts are significantly different at $p < 0.05$. $n = 3$, for Vitamin A ($\mu\text{g}/100\text{ g}$), vitamin C ($\text{mg}/100\text{ g}$) and vitamin E ($\mu\text{g}/100\text{ g}$), respectively for *Adenia cissampeloides* (ACD) and *Arthropteris Palisoti* (APD).

Mineral Content

The concentration ($\text{mg}/100\text{ g}$) of four essential minerals were determined in the goodies namely

magnesium (Mg^{2+}), sodium (Na^+), calcium (Ca^{2+}) and phosphorus (P) as shown in Table 3. Mg^{2+} levels of the goodies had no-significant differences between *Adenia cissampeloides* (ACD) between *Arthropteris Palisoti* (APD) at $p < 0.05$. However, the amount of Mg^{2+} in both ACD was considerably high relative to APD ($p < 0.05$). All the samples showed significantly ($p < 0.05$) low Na^+ content.

Table 3: Composition of mineral elements in delicacies

Delicacies	calcium ($\text{mg}/100\text{ g}$)	magnesium ($\text{mg}/100\text{ g}$)	phosphorus ($\text{mg}/100\text{ g}$)	sodium ($\text{mg}/100\text{ g}$)
ACD	69.03 \pm 2.20 ^a	74.7 \pm 2.36 ^a	30.63 \pm 0.80 ^a	10.13 \pm 0.25 ^a
APD	70.87 \pm 1.36 ^a	64.40 \pm 2.67 ^b	36.97 \pm 1.47	9.43 \pm 0.31 ^b

The trial groups with the same superscripts are not significant at $P < 0.05$ while test groups with non-identical superscripts are significant at $P < 0.05$. $n = 3$, for the concentrations of selected minerals ($\text{mg}/100\text{g}$) for *Adenia cissampeloides* (ACD) and *Arthropteris Palisoti* (APD),

Biological Evaluation (Food Intake)

The rats fed with the reference (casein) eat-up the highest quantity of food (g) next to those

fed with basal (protein-free). Although the difference was insignificant, the rats fed with *Arthropteris Palisoti* delicacy (APD) eat-up the least quantity of food. Details can be found in Table 4, they showed that the groups fed with trial delicacies (APD and ACD) recorded the lowest feed intake during the experimental period.

The trial groups with the same superscripts are not significant at $P < 0.05$, while the trial

groups with different superscripts are significant at $p < 0.05$. $n = 6$. For the food intakes (g) of rats fed with *Adenia cissampeloides* delicacy (ACD), *Arthropteris Palisoti* delicacy (APD), basal diet and Reference diet.

Table 4: Food Intake

Dietary groups	Food intake (g)
ACD	43.89 ± 14.34 ^a
APD	42.02 ± 7.98 ^a
BASAL	88.98 ± 10.61 ^{b,c}
REFERENCE	97.06 ± 14.70 ^c

Body Weight Variation

The body weight changes (g) of the rats during 10 days of the study are presented in Table 5. It was observed that there were no significant differences among the treated groups at $p < 0.05$

The trial groups with the same superscripts are not significant at $p < 0.05$. $n = 6$. The initial weight, final weight, and body weight change (g) of rats fed with *Adenia cissampeloides* delicacy (ACD), *Arthropteris Palisoti* delicacy (APD), and basal diet and Reference diet.

Table 5: The differences in body weight of the rats fed with reference, basal and test diets

Test diet	Weight before feeding	Weight after feeding	Change in weight
ACD	45.08 ± 1.96	47.89 ± 3.61	2.81 ± 2.08 ^a
APD	53.22 ± 3.83	57.30 ± 4.36	4.08 ± 3.21 ^a
BASAL	72.33 ± 1.87	75.17 ± 3.14	2.84 ± 1.65 ^a
REFERENCE	78.74 ± 2.16	84.18 ± 4.84	5.44 ± 3.05 ^a

Results had no difference among the groups treated when compare to the protein efficiency ratio (PER). Among the groups treated, no difference at ($p < 0.05$) was observed in the net protein utilization (NPU). Also, there was no significant difference in the net protein ratio (NPR) among the groups treated ($p < 0.05$). The true digestibility (TD) of the experimental animals had no observable differences among the groups treated at $p < 0.05$. The biological values (BV) of all the groups were significantly different from each other with the reference diet.

The trial groups without superscripts are different at $P < 0.05$. $n = 6$. For Protein efficiency ratio (PER), net protein utilization (NPU), net protein retention (NPR), true digestibility (TD), and biological values (BV) of rats fed with *Adenia cissampeloides* delicacy (ACD), *Arthropteris Palisoti* delicacy (APD), basal diet and Reference diet. The trial groups with the same superscripts are not significant at $P < 0.05$.

Table 7 showed that the fecal protein (FePr) content (%) among the groups were not significantly

Table 6: Biological evaluation of rats fed reference, basal and experimental diets

Test diet/analyzed indices	PER	NPU	NPR	TD	BV
ACD diet	0.28 ± 0.21 ^a	0.03 ± 0.02 ^a	0.23 ± 0.08 ^a	1.49 ± 0.41 ^a	2.37 ± 0.01
APD diet	0.41 ± 0.32 ^a	0.04 ± 0.03 ^a	0.35 ± 0.19 ^a	2.01 ± 1.60 ^a	2.18 ± 0.01
Basal diet	0.29 ± 0.16 ^a	-	-	-	-
Reference diet	0.54 ± 0.30 ^a	0.01 ± 0.00 ^a	0.36 ± 0.20 ^a	0.46 ± 0.32 ^a	3.03 ± 0.01

different from one another ($p < 0.05$). The carcass protein content (%) of the delicacies showed observable significant differences between groups at $p < 0.05$. The overall trend was in the decreasing order of *Adenia cissampeloides* delicacy (ACD), Basal feed, Reference feed and *Arthropteris Palisoti* goodie (APD) group.

Table 7: Fecal and carcass protein of trial rats

Test diets	Fecal protein (%)	Carcass protein (%)
ACD	11.71 ± 3.52 ^a	43.05 ± 3.46 ^a
APD	13.06 ± 4.95 ^a	34.10 ± 4.68 ^{b,c}
BASAL	12.89 ± 0.37 ^a	37.40 ± 8.25 ^b
REFERENCE	14.43 ± 1.25 ^a	35.60 ± 2.99 ^{b,c}

The trial groups with the same superscripts are not significant at $p < 0.05$, whereas the trial groups with different superscripts are significantly different at $p < 0.05$. $n = 6$. Fecal and carcass protein (%) of the rats fed with *Adenia cissampeloides* delicacy (ACD), *Arthropteris Palisoti* delicacy (APD), basal feed and Reference feed.

One mechanism by which proteins influence energy balance in the body is the regulation of appetite. Therefore, besides quantity, quality of food is an important feature in the modulation and regulation of appetite/satiety. Certain bioactive amino acid sequences have metabolic effects that influence appetite and satiety. Increased appetite is proportional to high food intake and vice versa.²⁸ As a "complete protein" laden with high concentrations of essential amino acids,²⁹ casein obtained from animal source has impacted positively on the appetite of experimental rats. This explains why the group feed with reference feed eat-up the highest quantity of feed among the groups that were treated. It is probable that the residual bitter taste apparently imparted on the delicacies by the wild edible vegetables due to the presence and activities of anti-nutrients³⁰ may have decreased the rats' appetite and make them overlooked vegetable-based delicacies relative to the reference and basal diets feed.

The changes in body weights of the net protein utilization (NPU), net protein retention (NPR), protein efficiency ratio (PER), and true digestibility (TD) of

the trial animals expressed non significant variation at ($p \geq 0.05$) among the groups treated. Reports about the proteins influences on body weight, regulation, and a decrease in the quality of proteins of foods correlate with lower body weights.³¹ The non-significant difference in the body weight of the groups suggests that the quality of proteins in the vegetable delicacies together with the growths rate of the rats were similar. This inference is strengthened by the non-significant differences in protein efficiency ratio, net protein retention, net protein utilization and true digestibility. These parameters are effective as useful determinants of protein quality. For instance, high PER values indicate efficient utilization of consumed proteins and considered as proportional to the high quality of proteins in foods. Protein utilization is dependent on amino acid composition and digestibility of proteins.³² The influence of dietary proteins relates to its quantity and relative proportion compared to other macronutrients. Some of the mechanisms which protein affects body weight are modulation/regulation of gluconeogenesis, thermogenesis and other metabolic functions.²⁸ Energy intake and expenditure requirements influence the metabolic utilization of proteins. The extent to which proteins provide vital amino acids and nitrogenous compounds for metabolisms in the body is an indication of proteins' quality. This can be estimated nitrogen balance in the animal. In addition, the ability to metabolize protein has been reported.³³

The test groups administered with the reference food (casein-based food) had the highest and lowest significant biological values (BV), respectively among the treated groups. This observation affirmed that animal's sources of proteins have higher BV than plant sources of proteins because of the high content of essential amino acids in the animal proteins.³⁴ According to studies, the high biological value is directly proportional to amounts of essential amino acids. Fundamentally, the same twenty amino acids make up both animal and plant proteins. The variation in the proportion of amino acids accounts for the differences between animal and plant protein.³⁵ However, several factors influenced food's biological value such as age, food matrix, sex, experimental duration and concentration of proteins in the food. The significant differences between delicacies ACD and APD biological values were due to the differential effects on the delicacies brought

about by food processing technique, heat treatments, and oxidation. They can induce the formation of Maillard compounds, oxidize amino acids and/or alter cross-linked peptide bonds which limit the bioavailability of amino acids and subsequently affect biological value.³⁶ Although, bioavailability is influenced by synergistic and potentiating actions of other food components, the existence of cofactors and intermediates promote the release of nutrients from the food matrix. The microstructure of processed foods and the formation of stable compounds enhanced the slow metabolic rate.³⁷ This result further corroborates the assertion that the amount of protein in a food plays a role in the food's biological value.³⁶

High fiber content affects the flow rate of food in the gastrointestinal tract and decrease the bioavailability of nutrients/energy.³¹ Undigested crude fiber, accompany by nutrients are usually excreted in feces which lower the digestibility, availability, retention, and utilization of nutrients such as proteins, vitamins and energy-related compounds.³¹ The fecal protein concentration is indicative of the quantity of consumed protein retained and utilized by the body. For instance, the higher the amount of protein utilization, the lower the concentration of protein excreted in the feces of the animal. This was reflected in high carcass protein levels because proteins were supposed to be retained and efficiently utilized to synthesize/repair body tissues with other functions.³⁸ The concentration of fecal protein between the groups showed no significant differences while the levels of carcass protein were similar between the groups. Therefore, it can be deduced that the administered foods had a similar effect on the rats. These novel vegetables can contribute its protein quality to enhance the balanced delicacy if applied.

Conclusion

Data in this study indicates that wild *Adenia cissampeloides* and *Arthropteris Palisoti*, vegetable delicacies contain valuable nutrients (though at varying concentrations) required by the body for its optimal functions. The delicacies could contribute effectively to the protein needs of the consumers. Their less impact on body weights suggests beneficial effects on weight-loss dietary regimens which ultimately improve the health status of the individual.

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Conflict of Interest

The author(s) hereby declare no conflict of interest on the publication of this article.

Authors' Contributions

The first author is the lead investigator, she carried out the animal study, laboratory/statistical analyses, second author wrote the draft manuscript and data interpretation, the third author designed the research while the fourth author carried out the literature search. All authors read and approved the final manuscript.

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