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Research Paper

Ethnopharmacological Use of .*Sphenostylis stenocarpa* (Hochst ex .A. Rich.) Harms Seed Milk As Alternative Medicine to Control Diabetes Mellitus In Streptozocin-Induced Rats.

[1]Nwankwo, Michael . O., [2]Ganyam, M. M.,[3] James, G. O.
[1] *Department of Biochemistry, ,College of Science, Federal University of Agriculture, Makurdi, Nigeria.*
[2]*Department of Biochemistry,College of Science, Federal University of Agriculture, Makurdi, Nigeria.*
[3]*Department of Biochemistry,College of Science, Federal University of Agriculture, Makurdi, Nigeria.*

Abstract:
Ethnopharmacy and the history of medicine have in common the description of medicinal systems, although the separation is geographical in the former case and historical in the latter case. However, Sphenostylis stenocarpa seed milk is an under-utilized legume seed milk that has been reported to have abundant bioactive compounds with hypoglycaemic activity. This study investigated the anti-diabetic activity of seed milk extract in Streptozocin-induced diabetic rats. The seed milk extract at a concentration of 100,200, 300 and 400 mg/kg body weight were orally administered to alloxan-induced diabetic rats for a period of fifteen (15) days. The oral glucose tolerance test was also carried out using animal experimental method. The phytochemical analysis of the milk revealed the presence of flavonoids, isoflavones, saponin, tannin, phytosterol, lignin and anthocyanidine at moderate concentrations. The acute toxicity test showed no lethality up to a concentration of 5000 kg/body weight. In oral glucose tolerance test, the S. stenocarpa seed milk exerted the highest response, similar to glibenclamide after 15 minutes and 30 minutes of administration compared with the control. The S. stenocarpa seed milk recorded the highest blood glucose- lowering effect after day 15 of treatment ($p < 0.05$) compared with the diabetic rats that were administered normal saline and 0.3 mg/kg body weight of glibenclamide. The seed milk of S. stenocarpa possessed anti-diabetic activity like the reference drug glibenclamide, and the results of this study revealed that the graded doses of the seed milk extract have blood glucose-lowering effect in a time and concentration-dependent manner.

Key words; *legume, herbs, phytochemicals, macronutrients, diabetes mellitus, bioactive compounds, ethnopharmacology.*

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I. INTRODUCTION

Ethnopharmacy was defined by {43} as the interdisciplinary Science that deals with the study of pharmaceutical, considered in relation to the cultural determinant that characterized the use of these means in a group. It consists of the identification, classification and categorization of acquired knowledge of the natural material from which the remedy will be produced (ethnobiology), preparation of the pharmaceutical forms (ethnopharmacy) ,and the socio- medical aspect implied in their uses. The use of herbs as remedy for the treatment of ailments is universal and continues to exist throughout the developing World {70}. More than 80% of the world'population uses herbs as their primary source of medication {48; 4}.With the assistance of the Agence de Cooperation Culture Technique (ACCT) in Paris, ethnobotanical Survey of the medicinal plants in 12 Francophone Countries in Africa were carried out {4} .To complement the efforts of ACCT, The Organization of African Unity's Scientific Technical Research Commission (OAU/STRC) recently started financing similar ethnobotanical survey in Anglophone African Countries . Such surveys were carried out in Western Nigeria in 1989 and Uganda in 1991, and the results had since been published by the OAU/STRC {4}. The ethnobotanical surveys of Tanzania and Somalia had also bean carried out. .*Sphenostylis stenocarpa* (Hochst ex .A. Rich.) Harms is a legume which belong to the family fabaceae.It is called African yam bean (AYB) and is distributed throughout tropical Africa {67; 68; 37}.Clinical studies have evaluated the effects of many different nuts and peanuts (legumes) on the management of Diabetes mellitus {6; 77; 79}. Diabetes is a complex, chronic illness requiring continuous medical care with multifactorial risk- reduction strategies beyond glycaemic control {79}. Legumes generally halt the tissue reactions of oxidation, inflammation, and vascular

reactivity {38; 50}.Thus, in addition to a favourable fatty acid profile, nuts and peanuts contain other bioactive compounds that explain their multiple Diabetic benefit s{50; 77}. Other macronutrients include plant proteins and fibres, and elements such as potassium, calcium, magnesium, as well as tocopherols and phytochemicals {75}. The observed anti-diabetic potentials of the seed milk of the African yam bean *(Sphenostylis stenocarpa*), and the fact that this raw material is of plant origin- legume send packing all the adverse drug reactions, contraindications and toxicities of chemotherapeutic anti-diabetics ,and finally appraised the specific aim of this study.

II. MATERIALS AND METHODS

Seed Collection, Identification and Preparation

The harvested seeds of *SphenostyIis stenocarpa* (African yam bean) were purchased from Ogige Market, Nsukka Enugu State, Nigeria.The seeds were identified authenticated by Mr. Alfred Ozioko and Prof.M.I. Uguru ,both of Centre for Ethnomedicine Drug Development (BDCP) and Crop Agronomy Department of the University of Nigeria Nsukka respectively. The seeds were washed with normal saline and oven-dried. They were de-shelled and some quantity fried at 30 -50^{0}C for milk-making..

Extraction of the AYB Seed milk

Five hundred grammes (500 g) seeds of AYB was roasted using frying pan for 50 minutes at 30-50^{0}C and were de-shelled when hot. The seed cotyledons were ground and sieved with fine-pored silk, and made in to milk by mixing and homogenizing in the ratio of 1:5 mass/volume of the flour/ de-ionized water .The prepared milk was used immediately for its storage always result to contamination and auto-oxidation of the labile substances.

Physicochemical analysis of the Milk

The extracted milk was subjected to sensory analysis by 20 Panelists invited from the the as shown in the results. The chemical properties was conducted by titration for acid value, and pH with pH metre and the presence of minerals was carried out using Atomic absorption spectrometer (AAS)(NARICT, Zaria) Nigeria.

Proximate analysis of the milk

The crude lipid were extracted using petroleum ether as solvent in a soxhlet apparatus and ash content (gravimetric by AOAC. The total carbohydrate was calculated by the difference method (sum of crude protein, ash, moisture and crude fat petroleum ether extract) minus the sum from 100). The moisture contents of the milk were determined after drying at 105 ^{0}C .

Phytochemical or Qualitative Analysis

The method described by {32} was used.

Test for Alkaloid; A quantity, 2millilitres of the milk extract had 8 millilitres of 1%HCl acid and a few drops of Dragenoff's reagent added. An orange precipitate was observed which indicates the presence of alkaloid. Also, 2 millilitres of the milk was had 8 millilitres of 1%HCl and a few drops of Mayer's reagent added. A creamy solution confirms confirms the presence of alkaloid.

Test for Saponin; A quantity 2 millilitres of the milk in a test tube was shaken vigorously for two minutes, frothing was observed. A quantity 1millilitre of the milk had 3 drops of olive oil added and was shaken vigorously, the disappearance of the frothing confirmed the presence of saponin.

Test for Flavonoid; A measured quantity: 3 millilitres of the milk had 4 milliltres of 1% $AlCl_3$ and methanol added to it .The appearance of yellow colouration indicates the presence of flavonoid. However, the addition of 4 millilitres of 1% KOH to 3 millilitres of milk showed a dark yellow colouration which confirmed the presence of flavonoid.

Test for Tannins; To a 2 milliltres of milk, add 10 millilitres of distilled water and 1% of FeCl, the appearance of a black colour ation confirms the presence of tannin.

Test for Cardiac Glycosides; A quantity 5 millilitres of the milk extract had 2 millilitres of glacial acetic acid, one drop of $FeCl_3$ and 1 millilitre of concentrated H_2SO_4 added to it. The appearance of orange colour shows the absence of cardiac glycosid**e**

Animal Protocol

A total of thirty five (30) male albino rats weighing180-220 grammes were used for the study, the rats were obtained from the faculty of Veterinary Medicine, University of Nigeria, Nsukka, Nigeria. The rats were divided into Six groups with five animals per group, and different treatments administered to each group;

Experimental Design

Group 1: Non- diabetic control (not induced).
Group 11:Streptozocin- induced diabetic rats administered 0.3 ml of normal saline.
Group 111: Streptozocin -induced diabetic rats administered with 0.3 mg/kg body weight of glibenclamide.
Group 1V: Streptozocin -induced diabetic rats administered 100 mg/kg body weight of Sphenostylis stenocarpa seed milk.
Group V Streptozocin -induced diabetic rats administered 200 mg/kg body weight of Sphenostylis stenocarpa seed milk.
Group vi Streptozocin -induced diabetic rats administered 300 mg/kg body weight of Sphenostylis stenocarpa seed milk.

Drug Preparation
Glibenclamide (Hovid,Ipoh Malayasia, Batch No. VUDIA 11 – 0, 5 mg) was purchased from a pharmaceutical shop in Nsukka, Enugu. The tablets were finely powdered and suspended in a normal saline and was filtered using a Buchner funnel and whatman no 1 filter paper at a concentration of 5 mg/ml and was administered at 50 mg/kg body weight.
Alloxan Monohydrate
An amount 1.0 gramme of Alloxan Monohydrate was dissolved in 20mls of normal saline (1.0/20ml) and the strength of the drug solution prepared was 100 mg/20 ml.
Induction of Diabetes
The animals were deprived of feed for twelve hours, and water maintained *ad lib* , after which 50 mg/kg b.w of freshly prepared alloxan monohydrate was administered intraperitoneally to the animals .
Animal Treatment
The experimental animals were treated in five different groups for 15 days. Group 111 was treated with the standard drug, while groups 1V, V and VI were treated with African yam bean milk of 5 mls , 10 mls, 15 mls and 20 mls dosage respectively, twice daily. The mean blood glucose levels in the animals were measured 72 hours after the drug administration by tail tapping method using Glucometer (ACCU – Chek, Active Roche Diagnostics). The experimental animals were treated with the standard drugs and the AYB milk by oral administration for 15 days and their mean blood sugar were recorded in their groups.

Glucose Level Determination
Procedure
a. The coding chip of the corresponding test strips to be used was inserted into the Accu-chek glucometer.
b. The area of the tail to be pricked was cleaned with swab containing methylated spirit and then pricked with a lancet.
c. The next step is the insertion of the test strip in to the glucometer.
d. A time, 2-4 minutes should be used for the activation of the strip in the glucometer after which, the blood sample was then dropped on the test area of the strip and the result displayed on the glucometer screen was recorded.

III. RESULTS

Physical Properties of African Yam Bean (AYB) Seed Milk

Colour	Sand brown
Aroma	6.8
Taste	7.4
Mouthfeel	7.2
Overall acceptability	7.5

Chemical Properties of AYB Seed Milk

Titratable acidity	26.22
Ph	5.2
Minerals by AAS	0.12605 ppm
Ca	0.05605ppm
Fe	0.0009ppm

Cu	
Pb and Cd	Not Detected

Proximate Composition of AYB Seed Milk

Nutrient	Relative Abundance (%)
Carbohydrate	59.32±1.02
Crude Protein	20.32 ± 0.06
Lipid	3.66 ± 0.14
Crude Fibre	8.3 ± 0.18
Ash	4.40 ± 0.04

Qualitative Phytochemical Analysis of AYB Milk

Parameters	**Relative abundance(mg/100ml)**
Isoflavones	++
Anthocyanides	+
Flavonoids	++
Saponins	++
Phytosterol	+
Lignin	+
Tannins	+

KEY: +: Low present: ++: moderate present.

The Seed milk of Sphenostylis stenocarpa was homogenized with de-ionized water in an electric blender (Nakai-462) China and used. The milk yield was observed to be 1.0 kg (33.3%).

In the experiment, there was no lethality or behavioural changes in the three groups of the mice that received 10, 100, and 1000 mg/kg body weight of the extract at the end of the first experiment. Based on this result, further increased doses of 1900, 2600 and 5000 mg/kg body weight of the milk showed that no death case was observed within 72 hours of administration. This result showed that the milk was safe at dose above 5000 kg body weight. The result of proximate analysis of AYB seed milk revealed the following: fibre (9.24 ± 0.18), carbohydrates (60.26 ± 1.02%), moisture (5.02 ± 2.04%), ash (3.40 ± 0.04%), crude protein (19.24 ± 0.06%), and lipids (2.84 ± 0.14%). The qualitative phytochemical analysis as observed in table 4 showed moderate presence of compounds such as isoflavones, flavonoids and saponin, while anthocyanidines, phytosterol, lignin and tannins were low in the sample.The results of the effect of *Sphenostylis stenocarpa* seed milk and glibenclamide on oral glucose tolerance in non- diabetic rat was shown in figure 1. The measured fasting blood glucose level reached its peak at 15 minutes after oral administration of glucose. Animals administered 2 g/kg body weight of glucose and 0.3 mg/kg body weight of glibenclamide had the highest significant ($p < 0.05$) reduction of fasting blood glucose concentration and sustained throughout all the measured time compared to the glucose level of other treatment groups. The animals administered 2 g/kg body weight of glucose and 100 mg/kg body weight of *Sphenostylis stenocarpa* seed milk showed significant ($p < 0.05$) decrease in blood glucose level 30 minutes from treatment compared to glucose level after 15 minutes of treatment, and also showed significant ($p < 0.05$) reduction in glucose after 45, 60, 90 and 120 minutes respectively compared to glucose level after 30 minutes of treatment. The animals administered 2 g/kg body weight of glucose and 0.03 ml of normal saline showed significant ($p < 0.05$) decrease in glucose level after 30 minutes compared to glucose level after 15 minutes and showed significant ($p < 0.05$) increase in glucose level after 60 minutes from treatment. The effect of *Sphenostylis stenocarpa* seed milk on glucose level of alloxan induced diabetic rats is shown in Figure 2. All animals induced with 150 mg/kg body weight of streptozocin showed significant ($p < 0.05$) increase in blood glucose level on day 0. Animals induced and treated with normal saline showed significant ($p < 0.05$) reduction in blood glucose level on day 3, 6, 9, 12, and 15 respectively. The seed milk of *S. Stenocarpa* possessed anti-diabetic activity like the reference drug glibenclamide, and the results of this study revealed that the graded doses of the seed milk extract have blood glucose-lowering effect in a time and concentration-dependent manner.

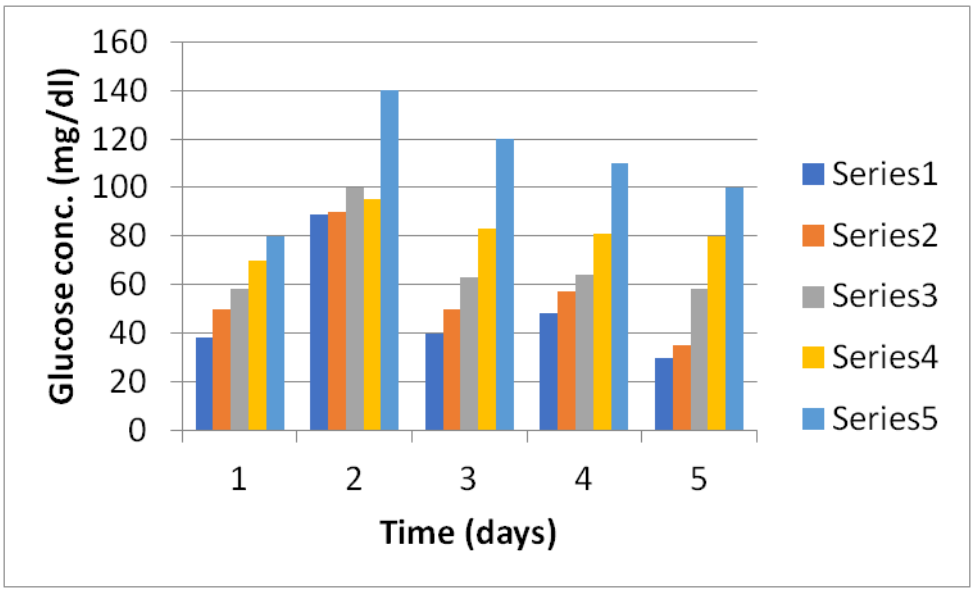

Figure 1: The effect of *Sphenostylis stenocarpa* seed milk on streptozocin- induced diabetes mellitus in Albino rats.

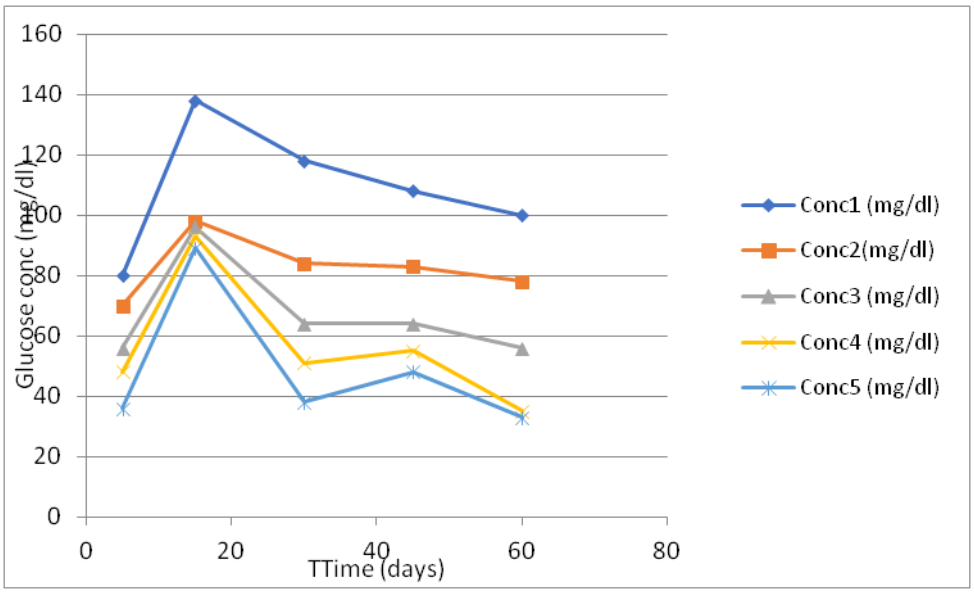

Figure 2: The effect *Sphenostylis stenocarpa* seed milk on oral glucose tolerance test of Albino Rats

IV. DISCUSSION

The study evaluated the use of the seed milk of African yam bean (*Sphenostylis stenocarpa*) from Kwande District of Benue State , North-Central Nigeria as an alternative medicine in the management of Diabetes mellitus. Twenty kilogrammes (20kg) mass of AYB was purchased from Saturday Market of Kwande Local Government Area of Benue state. Milk was extracted from 15kg mass of it , during which the percentage milk yield was . The physicochemical properties of AYB seed milk was found to be in agreement with other plants milk like soybean,, tigernut and coconut . The physical properties showed that the colour was sand brown, and other parameters that were sensorily tested by 20 panelists invited from the Department of Food Science and Technology scored the following; aroma- 6.80, taste -7.40, mouth feel-7.20 and overall acceptability-7.50 The results above was in agreement with work of {80} on physicochemical and sensory Characteristics of melon seed milk {15} on Soy- Coconut milk preparation {33} on physicochemical and sensory evaluation of Nigerian Tiger nuts {78: 14} who worked on the physicochemical properties of Tigernut-soy milk. Chemically, the titratable acidity of the AYB milk is 25.244 and a pH of 4.76. These agree with the work of {14} who also worked on the physicochemical properties of Tigernut---Soy milk mixture. The results of the proximate composition of AYB milk showed that the mean moisture value was 4.02± 2.04% dry weight. This result is somehow lower when compared with the mean moisture value of legumes ranging between 7.0%

and 11.0% as reported by {11}. However, this value is comparable to the moisture content of cashew nut flour (5.7%) {8; 9; 35} had earlier reported ash content of 5.0% for fruited pumpkins. The mean ash content of AYB milk in this study was 4.40 ± 0.04. This value is comparable to the value reported by {9} for other nuts. {65} had recommended that the ash content of nuts, seeds and tubers should fall in the range of 1.5-2.5% in order to be suitable for animal feed. In this study, the ash content of AYB milk was approximately within the range and may be recommended for animal feeds. The mean lipid value of 3.66 ± 0.14 reported in this study was relatively close to the values for other varieties of oil seeds ranging between 47.9-51.1% as reported by {35;12 ;27; 8} had earlier reported a mean lipid values of 49.2% and 47.01% respectively for pumpkin seed which is still closely related to the result obtained in this study. However, the value got in this study is comparably lower than the values of 36.7 as reported for cashew nut flour by {10} and 23.5% for soy bean by. Lipids are important in diets because they promote fat-soluble vitamins absorption {17}. It is also a high energy nutrient and does not add to the bulk in the diet. The crude proteins of AYB milk was found to be 20.32 ± 0.06. This value is low compared to the protein content of soy bean , cowpeas, pigeon peas, melon, pumpkin and gourd seeds ranging between 23.1-33.0% {59}. The implication of the protein level was that AYB milk can supply the recommended daily intake of protein for children {24}. Apart from the nutritional significance of proteins as source of amino acids, they also play a part in the organoleptic properties of food {57; 54; 55} the crude fibre of AYB milk was found to be comparable to other legumes with mean value ranging between 5-6% as reported by {9}. The maintenance of internal distension for a normal peristaltic movement of the intestinal tract is the physiological role which crude fibre plays {30; 3} reported that a diet low in fibre is undesirable as it could cause constipation and that such diets are usually associated with diseases of the colon like piles, appendicitis and cancer {9}. The value obtained for carbohydrates by difference is 59.32 ± 1.20 % . This value is a bit higher with an acceptable mean values for legumes, 20-60% of dry weight {11}. This result justifies the *Sphenostylis stenocarpa* as a possible rich source of energy and may be capable of supplying the daily energy requirement of the body {9} . Dietary flavonoids protect against cardiovascular diseases/diabetes mellitus. The emerging , consistent, and provable evidence suggests that flavonoids can improve endothelial functions and may reduce blood pressure and glucose level {32} through its vaso-relaxative effect on isolated arteries from rabbits as there is evidence that flavonoids metabolism is an important factor influencing the biological activity and effect of dietary flavonoids. Precisely, the flavonoid (Hesperidin) raises blood levels of HDL (good cholesterol) and lowers the LDL (bad cholesterol). It also prevents inflammation as well as relief pain {50}. The reductive effects in most of the cardiovascular parameters like systolic blood pressure, total cholesterol and blood glucose are the protective effects of saponin present in the seed milk of *Sphenostylis stenocarpa* .The presence of saponin resulted in the reduction of total cholesterol and inflammation {38}. Saponin, if regularly included in the diet may help the body protect itself from cancer and other cardiovascular diseases as saponin and saponin-like compounds have shown evidence that they can buttress the body's ability to fight cancer, diabetes mellitus and cardiovascular diseases {69}. Chemically, the titratable acidity of the AYB milk is 26.22 and a pH of 5.2. These agree with the work of {14} who also worked on the physicochemical properties of Tigernut-Soy milk mixture. Also, the atomic absorption spectroscopy (AAS) gave us the results of Calcium as -0.1260 Ppm, iron -0.05605 Ppm and Copper -0.0009 Ppm as the heavy metals Pb and Cd were not detected. Certainly, the above made the researchers convinced that the works of {75; 1; 6} as well as {74} on the nutritional qualities of this legume seeds gave the seeds the novel properties that may be the rationale behind the anti-diabetic potentials of this legume seed milk. The acclimatized an imals had diabetes induced to six of the seven groups. However, with the use of Accu- chek, Active Roche Diagnostics, we confirmed the induction of diabetes 30 minutes after the induction. The blood glucose level was orderly monitored in thirty rats using Accu-Chek, and in it, we were able to observe that the diabetic drug Glibenclamide has the ability of reducing the blood glucose in rats to an appreciable level. However, the African yam bean (AYB) milk showed more significant ($p < 0.05$) reduction of blood glucose than the Glibenclamide. The above blood glucose reduction pattern is in agreement with the work of {8;55,45, 53} who worked on the ethnobotanical study of plants used for the treatment of diabetes mellitus in the Eastern province of Southern Africa. Also, the mechanism of the blood glucose reduction by AYB milk agrees with Osinubu, Ajayi and Adesiyan,2006 in their evaluation of the anti-diabetic effect of aqueous leaf extracts of *Tripinanthus bufungil* in male spragne Dawley rats, in time and concentration-dependent manner. The cotyledon of AYB has a low glycaemic index and so, this may be the rationale behind the blood glucose reduction effect. The milk of *Sphenostylis stenocarpa* seed showed good anti-diabetic potentials and its mechanism of bood glucose reduction is concentration and time-dependent.

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Authors contribution

The authors confirm contribution to the paper as follows: the corresponding author conceptualized and, designed and wrote the paper, author number 2 performed the analysis and author number 3 contributed data or analysis tool.

Conflict of Interest The authors of this work has no conflict of interest whatsoever before, during and after this work.

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