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Exploring the Therapeutic Synergistic Intervention of *Ceriops decendra* with enriched protein of *Cyamopsis tetragonoloba* against Sreptozotocin Nicotinamide induced type-2 Diabetes

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Abstract

In this study the synergistic antihyperglycemic effect of the medicinal plant *Ceriops decendra* stem hydro-alcoholic extract (CD) and protein component isolated from *Cyamopsis tetragonoloba* seed (CT) was evaluated *in vitro* in α-glucosidase and α-amylase inhibitory bio-assays and *in vivo* against streptozotocin (STZ)-nicotinamide-induced diabetic rats with metformin as reference in a 28 days standard treatment protocol. The blood serum biochemical parameters and tissue (liver and kidney) antioxidative parameters were analysed. Histopathology of pancreas was also studied. The animal groups treated with the combination of both CD and CT have demonstrated a remarkable inhibition of α-glucosidase and α-amylase activities and restoration of diabetes-induced serum biochemical and tissue antioxidant parameters with improved pancreatic histology as compared to the above groups indicating the synergistic effect. The findings of present study infers that, combination of both CD and CT exerted a synergistic action exhibiting more potent antihyperglycemic effect indicating the beneficial role of protein component in management of diabetic complications.

Keywords: *Ceriops decendra*, gel electrophoresis, protein, diabetes, synergy.

Introduction

A concerned lifestyle disease called diabetes mellitus has grown into a problem on a global scale. According to the 2015 Diabetes Atlas published by the International Diabetes Federation (IDF), there are 415 million individuals worldwide who have been identified as having diabetes, or 8.8% among individuals aged 20 to 79. By 2040, it is expected that there will be more than 640 million adults worldwide who have diabetes. Since over 80% of all persons with diabetes who have not been diagnosed live in low- and middle-income countries, which account for 75% of the global population, the increase in numbers will be greatest there. China and India will have the largest increases in the number of diabetics among developing nations. Urban locations in developing economies have a higher rate of diabetes prevalence (1, 2). Rapid socioeconomic shifts brought on by industrialisation and urbanisation are the primary causes of the global diabetes epidemic, which is also largely driven by population expansion, poor lifestyle choices, and a lack of vigorous physical activity. Diabetes is a silent disease that can cause deadly and severe complications as well as raise expenditures. Nearly every system in the body is impacted by diabetes' long-term problems, but the eyes, kidneys, heart, feet, and nerves are particularly vulnerable. Anatomical,

structural, and functional alterations as a result of the micro- and macrovascular complications result in numerous organ dysfunction (3).

India is undergoing a shift in the prevalence of diabetes from urban to rural areas, the affluent to the less privileged, and older to younger individuals. India ranks second after China in the world in terms of the diabetes epidemic. In both urban and rural parts of India, the prevalence of diabetes has been rapidly rising. The most common form of diabetes is type 2, which is also the main cause of the diabetes epidemic in India. Type 1 diabetes is becoming more common in India as well. Type 2 diabetes prevalence increased gradually in the 1990s and significantly more quickly after 2000. Diabetes now affects more people than it did in 2000, when there were 32.7 million cases. compared to 35.5 million in 2003, in 2007 there were 40.9 million, 50.8 million people in 2010, 2013 saw 65.1 million, 69.2 million people in 2015, The predicted number in 2040 is 1.23 billion. The first study on the prevalence of diabetes in India was carried out in Calcutta (now Kolkata) in 1938. After verifying 96 300 medical records, it was discovered that 1% of the population had diabetes. Between 1973 and 2015, the prevalence of diabetes ranged from 1.1% in urban Lucknow to 25.2% in New Delhi. Few nationwide studies on the level of prevalence of this disorder have been conducted, and the majority of those studies used capillary fasting and a two-hour post glucose load to diagnose diabetes. The countrywide prevalence of diabetes has been reported to be 2.1% in the multi-centre study done between 1972 and 1975 by the Indian Council of Medical Research (ICMR) in six cities (Trivandrum, Calcutta, Cuttack, Delhi, Poona and Ahmadabad) and nearby rural areas (1, 4).

Physical, environmental, metabolic, chemical, and genetic elements all have a direct or indirect impact in the development and progression of diabetes. A diet heavy in antioxidant-rich fruits and vegetables lowers the incidence of diabetes, strongly indicating that these antioxidants may be useful diabetes-inhibiting agents (5). Protein consumption induces the release of glucagon, a hormone that aids in controlling blood sugar levels. In contrast to insulin, glucagon encourages the liver's stored glucose to be released, keeping blood sugar levels from falling too low. This system promotes glucose homeostasis and guards against hypoglycaemia. Consuming protein activates the hormone the insulin's release, which aids in the uptake and utilization of glucose by cells. For those with diabetes, especially type 2 diabetes patients who may have resistant to insulin, a sufficient protein intake maintains sufficient hormone synthesis and secretion (6).

Medicinal plants have long been known to serve as medicinal recourse to the mankind (7). *Ceriops decandra* (Griff.) Ding Hou (Rhizophoraceae) commonly known as Indian mangrove or spurred mangrove in English and *Garan* in Bengali is a shrubby mangrove tree widely distributed throughout the tropical Asia including India and Bangladesh. This plant has traditionally been used against diseases like ulcers, diarrhoea, dysentery, angina, boils, diabetes, hepatitis and wounds (8, 9). The objective of the present study is to explore the synergy effect of *Ceriops decendra* (CD) stem extract with the protein isolated from *Cyamopsis tetragonoloba* (CT) seeds against strepotozotocin nicotinamide-induced type 2 diabetes in rats.

Materials and methods

Extraction of Ceriops decendra stem

The plant *Ceriops decandra* (Griff.) Ding Hou (Rhizophoraceae) was collected on March 2022 from Jharkhali, South 24-Pgs., West Bengal, India and it was authenticated from the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India. The air-dried stem (250 g) was extracted with the hydro-alcoholic extraction by ethanol-water (70: 30) in a Soxhlet apparatus at a temperature 60-70°C. Then the solvent extract was then filtered in a member filter (Whatman paper). To exclude the solvent from the filtrate material a

solvent are then transfer into a rotary vacuum evaporator at a reduced pressure at 55°C. then concentrated sample were taken to a dryer for 24 Hour at 40°C then the extract was subjected to transfer in a lyophilise machine for 48 hours. The final yield was 13%.

Chemicals

All the chemicals used were of analytical grade obtained commercially.

Phytochemical screening

Preliminary phytochemical screening was performed on the foregoing extract to detect the presence of various phytochemicals (10).

Isolation of protein from Guar powder

Guar seed powder (procured from M/s Alona Pvt. Ltd.) was defatted by acid-alkali treatment followed by removal of phenolics and flavonoids as per previously reported protein acid salt alkaline methods (11). Then the resultant material was subjected to polyacrylamide gel electrophoresis followed by staining and de-staining. The identification of band are observed in the UV light at 254 and 366 nm and as the running capability depends upon the molecular weight of the sample, Here as a standard bovine serum albumin (BSA) has been used which molecular weight is about 65.5 Kda, its shows that it run up to the bottom of the gel, whereas the sample band run less compare to the standard it means it has higher the molecular weight based on the running capability its expected the molecular weight of the band are lies in between 75-80 Kda (12). Purity of isolated protein was assessed by the micro-Kjeldahl method (83%).

Animals

Adult Wistar albino rats of either sex were used. The animal was feed with the normal diet for 5 Days in a animal house in acclimatize condition relative humidity 55%, temp. 22±5°C. The experimental procedure started after review and the approval by the IAEC reference no. JU/IAEC-22/43.

In vitro antioxidant activity

The radical scavenging or antioxidant activity of DPPH is determined by its capacity to receive an electron. Strong antioxidants will rapidly transfer an electron to DPPH, significantly reducing the amount of purple that DPPH exhibits. The amount of colour change is related to the tested compound's antioxidant strength (13).

Prepare a stock solution with a 0.1 mM suggested concentration by dissolving the DPPH powder in a suitable solvent (such as methanol or ethanol). Weight 1.97 gram of DPPH in 50 ml of methanol. Molecular weight of DPPH 394 gram/mol. Make careful to thoroughly mix the DPPH powder until it dissolves. Cover the container with aluminium foil and store the DPPH solution in an amber bottle to shield it from light.

One well should be designated as a blank control that solely contains the solvent and DPPH solution. This well will assist in making any background absorbance corrections. 100 µL of each sample or antioxidant standard should be added to the appropriate microplate wells. Add 100 µL of the DPPH solution to each well containing the samples or standards. Set the timer for 30 minutes and allow the plate or cuvette to incubate there in the dark and at room temperature. During this period, the samples' antioxidant chemicals will interact with the DPPH radical. Utilising a spectrophotometer, determine each well's absorbance following the incubation time at a certain wavelength, commonly 517 nm. Utilise the blank control effectively to clean the spectrophotometer.

In vitro alpha amylase and alpha glucsidase inhibitory assay

Alpha-amylase is an enzyme involved in the digestion of carbohydrates, and the alpha-amylase inhibitory test measures a sub-

stance's capacity to block its activity.

Preparation of enzyme solution: 1 mg of enzyme solution = 30 unit of enzyme.

For the preparation of 1 unit/ ml of enzyme solution take 1 mg of enzyme and dissolved it in the 30 ml of buffer. And then store it in -20 Degree Celsius for the further use of the enzyme.

Preparation of starch substrate

1% W/V 100 mg of starch dissolved in 10 ml of buffer solution and shake it gently and dissolved it in a hot water contact and shake the system over and over again.

Preparation of 5.3 M Potassium sodium tetrahydrate solution

Dissolved 14.96 gram (PST) in 10 ml 2 M NaOH solution. And before that prepare 2 M NaOH solution by dissolving 1.6 Gram of NaOH are dissolved in 20 ml, of distilled water.

Prepare of 96 mM DNSA solution: 21.9 mg/ml 438 mg of DNSA in 40 ml of buffer solution.

Sample preparation: prepare the stock solution of test sample and in different concentration right from to lower to upper conc. 10,20,40,60,80, 100 µg/ ml .

Procedure: 30 µl of phosphate buffer or plant extracts Acarbose was used as a positive control after 30 µl of -amylase enzyme solution was combined with it and pre-incubated at 37°C for 10 minutes. Following this, 40 µl of starch solution (the substrate) was added, and the reaction was started at 37 °C for 30 minutes. The stop reagent, 20 µl of DNSA solution, was then added and heated in a water bath for 5 minutes. The mixture was diluted with 80 µl of buffer and warmed to room temperature. At 540 nm, the mixture's absorbance was measured using a Spectramax ID3 spectrophotometer (4).

In vivo study for anti-diabetic activity

For the experiment in each group 6 animal was selected which has average body weight more than = 170 gram

Initially 110 mg/kg nicotinamide was injected then wait for 20 min after all 40 mg/kg streptozotocin (STZ) was injected. Then the animal was provided dextrose water for three days after three days blood glucose was measure.

No significant raise of blood glucose Average blood glucose was – 125 mg /dl. Then again animal was feed for five days with the normal diet when the animal become healthy then 35 mg/dl STZ was injected.

After three days blood glucose measure, average blood glucose of each group. Blood glucose was measure in a regular interval (5 days) interval

Fasting Blood Glucose measurement in a regular interval

Group- A Standard control (Metformin) 50 mg/kg

Group-B Diabetics control (Nicotinamide + STZ) Induced

Group -C Normal control (Vehicle control)

Group-D Test control *Ceriops decendra* extract (Nicotinamide + STZ induced) CD extract 250 mg/kg)

Group-E Test control CD+CT (Nicotinamide + STZ induced) CD 250 mg/kg + CT 0.9 gram /kg enriched protein

After 28 days of foregoing treatments the animals were sacrificed and the serum biochemical parameters and tissue (liver and kidney) antioxidative parameters were analysed by the fully automated Auto-Analyser SELECTRA. Histopathological study of pancreas was performed at 40x (2, 6).

Statistical analysis

For the analysis of the mean blood glucose level of various group Two-way ANOVA group analysis use for the measurement of mean difference between and within the group (Mean ± SD). The analysis shows there is significant different between test control supplement with the extract group ***P < 0.0001 compare

to the *Ceriops decendra* dose group and the alpha value = 0.05 compare to the standard treatment group and significant diff. among means. It means the average blood glucose of the combinational drug is much more effective compare to the drug extract along or the standard treatment.

Results and Discussion

Table 1: Preliminary phytochemical test of *Ceriops decendra* and *Cyamopsis tetragonoloba* (+) presence (-) Absent.

Sl. No.	Test Name	Ceriops decendra	Cyamopsis tetragonoloba
1	Flavonoids	++	-
2	Alkaloids	++	+
3	Phenol	++	-
4	Saponin	-	-
5	Tannin	+	-
6	Glycoside	+	+
7	Protein	-	+++
8	Terpenoids	++	--

Table 2: *In vitro* antioxidant assay.

Concentration µg/ml	Control	Sample	%RSA	IC50 Value
50	0.521	0.312	40.11516	1.709
100	0.510	0.192	62.35294	5.855
150	0.521	0.122	76.58349	10.002
200	0.452	0.067	85.17699	14.149
250	0.512	0.045	91.21094	18.295
300	0.511	0.021	95.88235	22.442

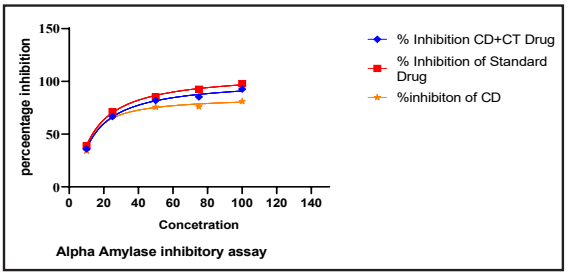

Figure 1: Alpha Amylase inhibitory assay. IC_{50} value of CD+CT= 7.554 & Standard= 8.186 And IC_{50} value of CD is = 9.12.

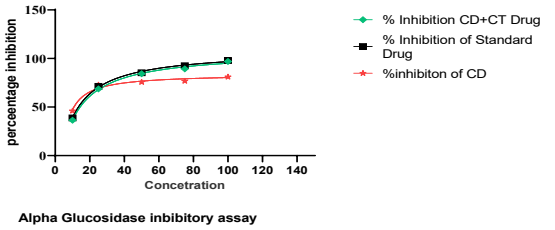

Figure 2: Alpha Glucosidase inhibitory assay. IC50 value of CD+CT= 7.152 Std= 7.819 CD IC50 = 8.219.

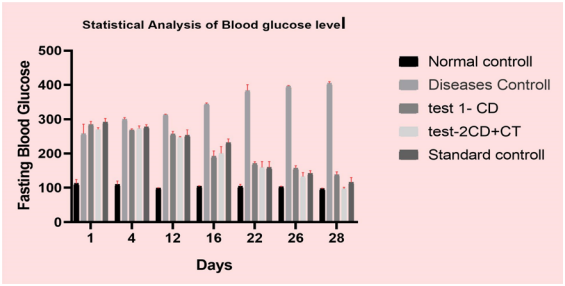

Figure 3: Antidiabetic effect of plant extract and its combination with protein.

Table 3: Oral glucose tolerance test.

Fasting Blood Glucose (Group-A) *Mg/dl*	Fasting Blood Glucose (Group-B) *Mg/dl*	Fasting Blood Glucose (Group-C) *Mg/dl*	Fasting Blood Glucose (Group-D) *Mg/dl*	Fasting Blood Glucose (Group-E) *Mg/dl*
298 ±2	298±7	110±8	299±4	300±8
285±3	314±5	102 ±7	286±3	285 ±2
265 ±2	325±3	105 ±5	*268*±4	*272* ±4
191±4	346±4	106±4	193 ±6	161±3
165±5	372±2	112±3	168±4	163±4
145±3	410±3	108 ±4	152 ±3	152 ±2
133±5	418±4	112±2	142 ±5	131 ±3

Table 4: Serum biochemical parameters.

Test Name	Standard Control	Diabetics control	Normal control	Test control *Ceriops decendra*	Test control CD+CT
Total cholesterol	50± 5mg/dl	68± 3mg/dl	48±2mg/dl	53±8mg/dl	42±6mg/dl
Total Protein	90±2 g/l	115±6 g/l	95±3 g/l	126± g/l	115±g/l
SGPT	38±2 U/l	72±8 U/l	35± u/l	52±6 u/l	41±u/l
SGOT	44±3 IU/l	83±3 IU/l	38±2 IU/l	63±3 IU/l	51±2 IU/l
ALT	162±2IU/l	182±3IU/l	150±5IU/l	175±3IU/l	164±3IU/l
AST	35±3IU/l	65±3IU/l	46±3IU/l	42±3IU/l	55±3IU/l
Bilirubin	4.8±0.5mumol/l	7.8±0.5mu mol/l	3.8±0.2mu mol/l	2.8±0.3mu mol/l	3.8±0.4mu mol/l
HbA_1C	7.2±0.5	9.2±0.3	6.2±0.4	7.2±0.2	6.8±0.3
HDL	35±2 mg/dl	25± mg/dl	38±3 mg/dl	34 ± 2 mg/dl	31±5mg/dl±
LDL-C	78±3 mg /dl	91±2 mg /dl	70±3 mg /dl	88±2 mg /dl	80±4mg /dl
Urea	78.70±8 mg/dl	112.50±3mg/dl	65.70±2 mg/dl	88±8 mg/dl	75.70±8 mg/dl

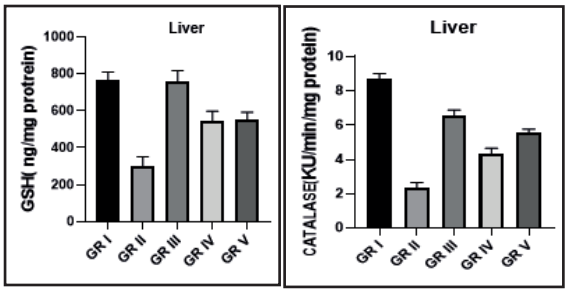

Figure 4: Hepatic antioxidant parameters. P-Value <0.001, with 95.00% CI of diff., Mean Diff. 2.052, SE of diff. 0.1109

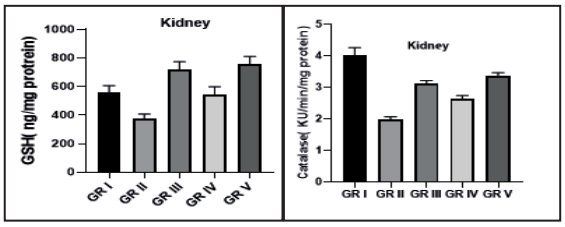

Figure 5: Renal antioxidant parameters. P-Value <0.0001, SE of diff. 41.30, Mean Diff. 466.6 Compare to negative control and p** compare to the standard and followed by the treatment, CI of diff. 95.0%

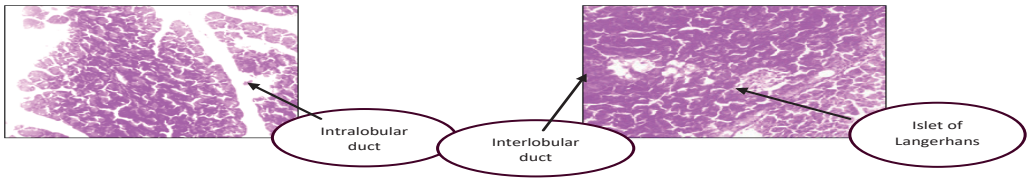

Fig. 6A: CD 250 mg/kg dose pancreatic beta cell the density of the beta-cell is less s compare to standard or the supplementary dose group. (Magnification - 10x40)

Fig. 6B: Test control Treatment CD + CT treatment the cluster beta cell- can be seen the test supplementary group and cell are dense. (Magnification - 10x40)

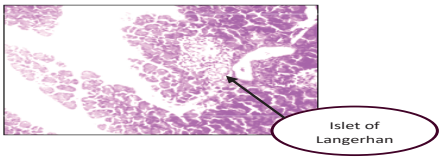

Fig. 6C: Negative control Nicotinamide +STZ induced; Here the number of beta-cell count decrease Gradually
(Magnification - 10x40)

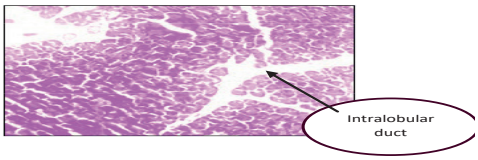

Fig. 6D: Another picture of CD 250 mg/kg treatment pancreas the density of the cell is less compare to the standard and supplementary dose group animal pancreas (Magnification - 10x40)

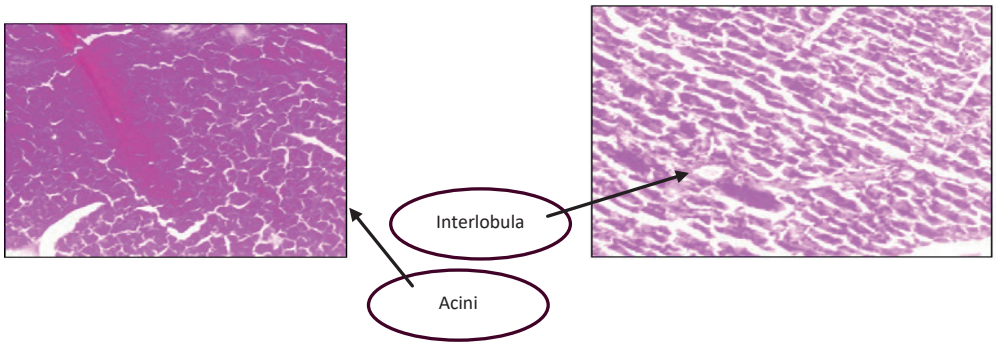

Fig. 6E: Normal control Without diabetes here the density of the cell is normally distributed (Magnification - 10x40)

Fig. 6E: Standard control Metformine dose 50 mg/kg (Magnification - 10x40)

Figure 6: Pancreatic tissue histopathology.

Discussion

Prevalence and co-morbidity in Type-2 diabetes is quite higher, currently. Synthetic molecule uses which targeting the many pathways, has decorates consequence in long term, Medicinal plants contain various phytochemicals which are responsible for the therapeutics benefits (14). In the present experiments the sole objective was to identify the effect of protein component over pre-clinical study with the plant extract of a mangrove plant *Ceriops decendra*. This mangrove plant has their several implications on therapeutics in-terms of traditional use. On the other hand, the protein component are used widely now a days as supplementary component for the treatment of diabetics patient. *Ceriops decendra* used for the treatment of diabetics traditionally and the protein component has their significant role on the metabolism process. Here in this study, we have evaluated the effect of foregoing plant extract with the enriched protein *Cyamopsis tetragonoloba* on pre-clinical study type-2 diabetes.

Right from the collection and identification of the sample here we have evaluated the comparative effectiveness and the enzyme-inhibitory assay. With solvent enrichment method purification of protein was carried out then it was supposed to quantify the enriched protein the purification was about 83%, analysis was carried out by the micro-Kjhadal method. After purification of the protein, we have assessed the anti-oxidant and in-vitro alpha-amylase inhibitory assay with combination of drug extract and the purified protein which show very promising result as compare to the plant extract along. In the *in vitro* level we have analyse the combinational anti-oxidant activity DPPH assay and the comparative enzyme inhibitory assay (15). The enzyme assay was quite surprising on the combination result its work as close to the standard drug acarbose. The IC50 value for the combination of isolated protein and the extract was 7.152 whereas the reference drug IC50 value 7.819 and *Ceriops decendra* is 8.219 which is quite differ from each other for the alpha-glucosidase enzyme. On the other hand, the enzyme inhibitory assay alpha – amylase has their own way of inhibitory potency the IC50 value of different drug and drug combination as follows IC50 value of CD+CT= 7.554 and standard = 8.186 and IC50 value of CD is = 9.12. In *in vivo* study, there were five group in there after the 28 days dose treatment each of the group except the normal control and the negative control group the mean blood glucose level after the 28 days which shows significantly lower as compare to the standard and the only extract treatment group. Significance on the statistical

analysis the p value is < 0.001 with the 95% confidence interval.

Overall, the tissue anti-oxidant parameter is then use to analyse the effect of each and individual group of their effect on the anti-oxidant property. In every anti-oxidant parameter, the combination supplementary dose group shows good effect on GSH and catalase activity and the statistical analysis ANOVA was conducted to determine the significance level of those individual group.

The experiment has fewer limitation that the assessment of the proper protein band is quite important due improper estimation of the band can lead to wrong interpretation of the molecular weight detection. During the experiment it was an little challenge to understand the protein band here after the gel electrophoresis this limitation need to assess again for to make the experiment robustness. As the protein plays a greater role on the metabolic dysfunction a proper amount of protein specially essential and semi-essential has greater impact on the metabolic regulation directly and indirectly. Based on the above experiment here we need to make robustness of the data by the multiple time study and look the importance of the natural occurring plant supplement for the treating the diabetes. Further studies in this way can yield an effective natural regime against diabetes.

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Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Dash S, Pattnaik G, Kar B, Sahoo N, Bhattacharya S. An approach towards method development to investigate the anti-diabetic activity on experimental animals. Curr Trends Biotechnol Pharm 2021; 15: 330-348.
2. Dash S, Sahoo N, Pattnaik G, Ghosh G, Rath G, Bhattacharya S, Kar B. Antihyperglycemic effect of *Annona squamosa* leaf and oleanolic acid combination in diabetic albino rats. Curr Trends Biotechnol Pharm 2023; 17: 1004-1012.
3. Martinez-Sanchez A, Sałowka A. Molecular mechanisms of nutrient mediated regulation of microRNAs in pancreatic β-cells. Front Endocrinol 2021; 12: 704824.
4. Patra S, Bhattacharya S, Bala A, Haldar PK. Antidiabetic effect of *Drymaria cordata* leaf against streptozotocin–nicotinamide-induced diabetic albino rats. J Adv Pharm Technol Res 2020; 11: 44-52.
5. Biswas M, Kar B, Bhattacharya S, Kumar RBS, Ghosh AK, Haldar PK. Antihyperglycemic activity and antioxidant role of *Terminalia arjuna* leaf in streptozotocin-induced diabetic rats. Pharm Biol 2011; 49: 335-40.
6. Kumari R, Goldar WA, Mondal S, Patra S, Bhattacharya S, Haldar PK. Protective effect of *Basella alba* leaf against diabetic nephropathy in rats. Adv Tradit Med 2021; 21: 111-119.
7. Bhattacharya S. A review on experimentally proven medicinal plants and their constituents against fluoride toxicity. J Environ Pathol Toxicol Oncol 2023; 42: 51-64.
8. Nabeel MA, Kathiresan K, Manivannan S. Antidiabetic activity of the mangrove species *Ceriops decandra* in alloxan-induced diabetic rats. J Diabetes 2010; 2: 97-103.
9. Mahmud I, Shahria N, Yeasmin S, Iqbal A, Mukul EH, Gain S, Shilpi JA, Islam MK. Ethnomedicinal, phytochemical and pharmacological profile of a mangrove

plant Ceriops Decandra GriffDin Hou. J Complement Integr Med 2018; 16: 2017-0129.

10. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. Int J Chem Studies 2020; 8: 603–608.

11. Asati V, Sharma PK. Purification and characterization of an isoflavones conjugate hydrolyzing β-glucosidase (ICHG) from *Cyamopsis tetragonoloba* (Guar). Biochem Biophys Rep 2019; 20: 100669.

12. Ladner-Keay CL, Turner RJ, Edwards RA. Fluorescent protein visualization immediately after gel electrophoresis using an in-gel trichloroethanol photoreaction with tryptophan. Meth Mol Biol 2018; 1853: 179–190.

13. Bhattacharya S, Haldar PK, Zaman MK. Anti-inflammatory and *in vitro* antioxidant property of *Zanthoxylum nitidum* root. Curr Trends Biotechnol Pharm 2010; 4: 774-783.

14. Bhattacharya S. Antineoplastic potential of *Trichosanthes dioica* root: A treatise. Indian J Nat Prod Resour 2023; 14: 202-209.

15. Bhattacharya S, Haldar PK, Zaman MK. Anti-inflammatory activity and antioxidant role of *Zanthoxylum nitidum* bark. Orient Pharm Exp Med 2011; 11: 271-277.