ANTIDIABETIC EFFECT OF ACANTHOPHORA SEAWEED- In Vitro Study

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ABSTRACT

INTRODUCTION - Seaweed extracts and its bioactive compounds have antidiabetic potential as they inhibit carbohydrate hydrolyzing enzymes in vitro and exhibit blood glucose lowering effect in random and post-prandial blood glucose tests in vivo. In addition, they have been associated with reduced weight gain in animals probably by decreasing mRNA expression of pro-inflammatory cytokines with a concomitant increase in mRNA expression levels of anti-inflammatory cytokines. However, the detailed and in-depth studies of seaweeds as whole, their bioactive isolates and their extracts need to be explored further for their health benefits and wide application in food, nutraceutical and pharmaceutical industries.

AIM & OBJECTIVE - The present study aims to focus on the antidiabetic, effect of the seaweed *Acanthophora* .sp through its methanol extract.

MATERIALS & METHODS - Seaweed samples of ACANTHOPHORA were collected from the Rameshwaram Gulf Biosphere Reserve coastal areas.

Collected seaweed samples were washed extensively with fresh water and then extracted with methanol (Weight/volume). The methanol extract was dried
and powdered. The dried extract was then used for biological screening.

Alpha-amylase inhibitory effect , pancreatic lipase inhibitory assay, and inhibition of DPP assay were carried out.

RESULT - All the assay results were highly significant when compared to that of standard drugs. This shows that Acanthophora has significant antidiabetic role in reducing blood sugar management.

CONCLUSION - Acanthophora extract has significant antidiabetic efficacy through preliminary experimental results. Further research on this through in-vivo experiments will lead to the isolation of drug candidates for advanced biomedical research.

KEYWORDS - Acanthophora seaweed , In vitro , Antidiabetic effect , Seaweed extract , Bioactive compounds , Diabetes mellitus

INTRODUCTION

Red seaweed Acanthophora is a member of the Rhodomelaceae family and is found in many coastal regions of the world. Because of its possible health benefits, researchers and practitioners of traditional medicine have given this marine macroalgae a lot of attention. Studies on the medicinal qualities of Acanthophora seaweed, in particular its in vitro antidiabetic effects, have been conducted recently. Chronic metabolic disease known as diabetes mellitus is typified by hyperglycemia brought on by insufficient insulin secretion or insulin resistance. Globally, the prevalence of diabetes is rising, which emphasizes the critical need for new and efficient treatment approaches. (1,2)

The identification of naturally occurring compounds possessing antidiabetic qualities has sparked curiosity as possible substitutes or supplements to traditional diabetes drugs. Seaweed extract from Acanthophora is a rich source of bioactive substances with potential biological effects, such as proteins, lipids, polysaccharides, and polyphenols. Studies have suggested that these compounds could be important in reducing hyperglycemia and the problems that come with it, which is why Acanthophora seaweed is a good option for managing diabetes. (3,4)

Acanthophora seaweed extract contains a substantial amount of polysaccharides, which have been thoroughly studied for their possible antidiabetic properties. These complex carbohydrates have a variety of structural and functional characteristics that could affect insulin sensitivity and glucose metabolism. Acanthophora seaweed polysaccharides have been shown in vitro to inhibit α-amylase and α-glucosidase, two important enzymes involved in the digestion and absorption of carbohydrates. Acanthophora seaweed extract may lessen postprandial glucose spikes and aid in glycemic control by inhibiting the activity of these enzymes. (5)

Moreover, it has been demonstrated that Acanthophora seaweed extract improves the uptake of glucose by adipocytes and skeletal muscle cells. Increased expression and translocation of glucose transporter proteins, like GLUT4, to the cell membrane mediate this improved uptake of glucose. As a result, Acanthophora seaweed extract may enhance peripheral tissue glucose uptake and insulin sensitivity, thereby preventing insulin resistance, a feature of type 2 diabetes. (6)

The anti-inflammatory qualities of Acanthophora seaweed are another fascinating facet of its potential as an antidiabetic agent. Chronic low-grade inflammation plays a major role in the pathophysiology of diabetes and its complications. By upregulating anti-inflammatory cytokines like IL-10 and downregulating pro-inflammatory cytokines like TNF-α and IL-6, Acanthophora seaweed extract has demonstrated anti-inflammatory activity. This immunomodulatory effect

may lower the risk of inflammatory diabetic complications and aid in overall glycemic control. (7)

Diabetes mellitus is a metabolic disease that has been present for as long as humans have, and it is believed to be incredibly common worldwide. It also has a substantial financial impact due to its substantial contribution to hospitalization and disability. There is currently no known treatment for diabetes mellitus, which calls for strict management. The situation was especially bad in developing countries like India, where diabetes has become an unfortunate side effect of unprecedented economic growth. It was strongly supported by higher than ever incidences in clinical and experimental swot. It claims that oxidative stress resulting from hyperglycemia plays a significant role in the onset of diabetes mellitus (DM). (8)

Diabetes is commonly associated with hyperglycemia, significant amplification of reactive oxygen species (ROS), and decreased coordination of antioxidant defense. Oxidative stress, an episode of oxidant factors over antioxidant systems, plays a fundamental role in the pathophysiology, development, and complications of diabetes. Consequently, it makes sense that a drug that has been shown to reduce oxidative stress in vivo would also slow the progression of cell damage in clinical diabetes. (9)

Rich in fruits, vegetables, and plants, dietary flavonoids have been associated with several potentially beneficial effects in the prevention and treatment of oxygen-related diseases, including better glucose utilization in type II diabetes and a lower risk of developing diabetic hiccups.Current research in this field is illuminating possible benefits for diabetes. (10)

Seaweeds have long been used by the coastal working class in many countries as a food source and a traditional remedy for gout, eczema, and helminthes infections. Seaweeds' potential anticancer properties have garnered a lot of attention recently, owing to their abundance in phyto-constituents. It has been suggested that pancreatic beta-cells may be impacted by raw Swedish seaweed or its organic extracts. (11)

The aim of the topic is to explore the in vitro antidiabetic effects of Acanthophora seaweed extract. The objective is to investigate the potential of the extract's bioactive compounds, such as polysaccharides and polyphenols, in mitigating hyperglycemia, enhancing glucose uptake, and alleviating oxidative stress for diabetes management.

MATERIALS & METHODS

Sample collection and extraction:

Seaweed samples of Acanthopora were collected from the Rameshwaram gulf biosphere reserve coastal areas (Lat’ 9.2876° N, Long’ N: 79.3129° E). Collected seaweed samples were washed

extensively with fresh water and then extracted with methanol (Weight/volume). The methanol extract was dried and powdered. The dried extract was then used for biological screening.

Alpha-amylase inhibitory effect:

α-amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification. In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha-amylase (1 U/ml), and 20 µl of varying concentrations of Acanthopora extract and fractions (0.1, 0.2, 0.3, 0.4, and 0.5 µg/ml) was pre-incubated at 37°C for 15 min. Then, 20 µl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na2 CO3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using Multiplate Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

$$\text{Inhibitory activity (\%)} = (1 - As/Ac) \times 100$$

Where,

As is the absorbance in the presence of test substance and Ac is the absorbance of control.

Pancreatic lipase inhibitory assay:

Porcine pancreatic lipase (PPL, type II) activity was measured using p-nitrophenyl butyrate (p-NPB) as a substrate. PPL stock solutions (1 mg/mL) were prepared in a 0.1 mM potassium phosphate buffer (pH 6.0) and the solutions were stored at −20 °C. To determine the lipase inhibitory activity, the Acanthopora extracts (final concentrations 0.1,0.2, 0.3, 0.4, 0.5 µg/mL) or Orlistat (at same concentrations) as a positive control were pre-incubated with PPL for 1 h in a potassium phosphate buffer (0.1 mM, pH 7.2, 0.1% Tween 80) at 30 °C before assaying the PPL activity. The reaction was then started by adding 0.1 µL NPB as a substrate, all in a final volume of 100 µL. After incubation at 30 °C for 5 min, the amount of p-nitrophenol released in the reaction was measured at 405 nm using a UV-Visible spectrophotometer. The activity of the negative control was also examined with and without an inhibitor.

Inhibition of DPP IV assay:

The inhibition of DPP IV activity was performed using the enzyme protocol (Abcam DPP IV screening kit) with slight modification. Sitagliptin was used as the standard inhibitor. Briefly, 30 µL of the buffer solution, 10 µL of enzyme DPP IV, 10 µL of Acanthopora extracts (0.1,0.2, 0.3,0.4, 05µg/ml) and 50 µL Gly-Pro-AMC as the substrate, was added into the well. The mixture was shaken and incubated for 30 min at 37oC to have the complete reaction. In control

wells, the inhibitor was replaced by aquabidest. The fluorescence of free AMC group was measured on excitation wavelength 350-360 nm and an emission wavelength 450-465 nm by using a microplate reader Percentage of inhibition was calculated using the formula:

% Inhibition Initial Activity Inhibitor

$$\text{Initial Activity} = - \times 100$$

RESULT

Acanthopora exhibited highly significant alpha glucosidase inhibitory effect when compared to the standard drug Acarbose. As percentage of alpha glucosidase inhibition is gradually increasing with increasing concentrations and maximum inhibitory effect of IC50 at 0.4 mg/mL was observed as equal with that of standard drug. In the same way Acanthopora inhibits the pancreatic lipase and DDP-IV factors which are main diabetic contributing agents. Acanthopora inhibits pancreatic lipase with minimum IC50 value of 0.2µg/mL and IC50 value of 0.4982µg/mL for DPP-IV. All the assay results were highly significant when compared to that of standard drugs. This shows that Acanthopora has significant antidiabetic role in reducing blood sugar management.

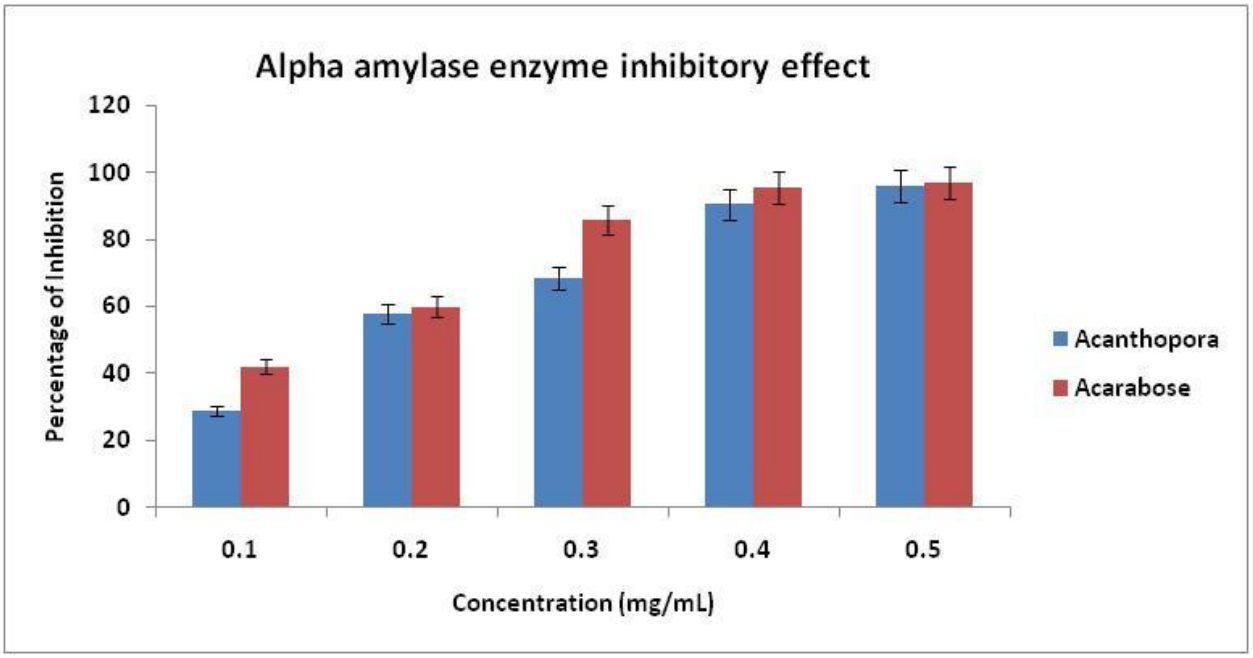

Table 1 Alpha amylase inhibitory effect.

In this graph, we have taken x axis as concentration of the substance and y axis as percentage of inhibition of the substance. We have taken acarbose as standard and saw the effect of our

substrate . The result shows that our extract shows maximum inhibitory effect of IC50 at 0.4 mg/mL was observed as equal with that of standard drug.

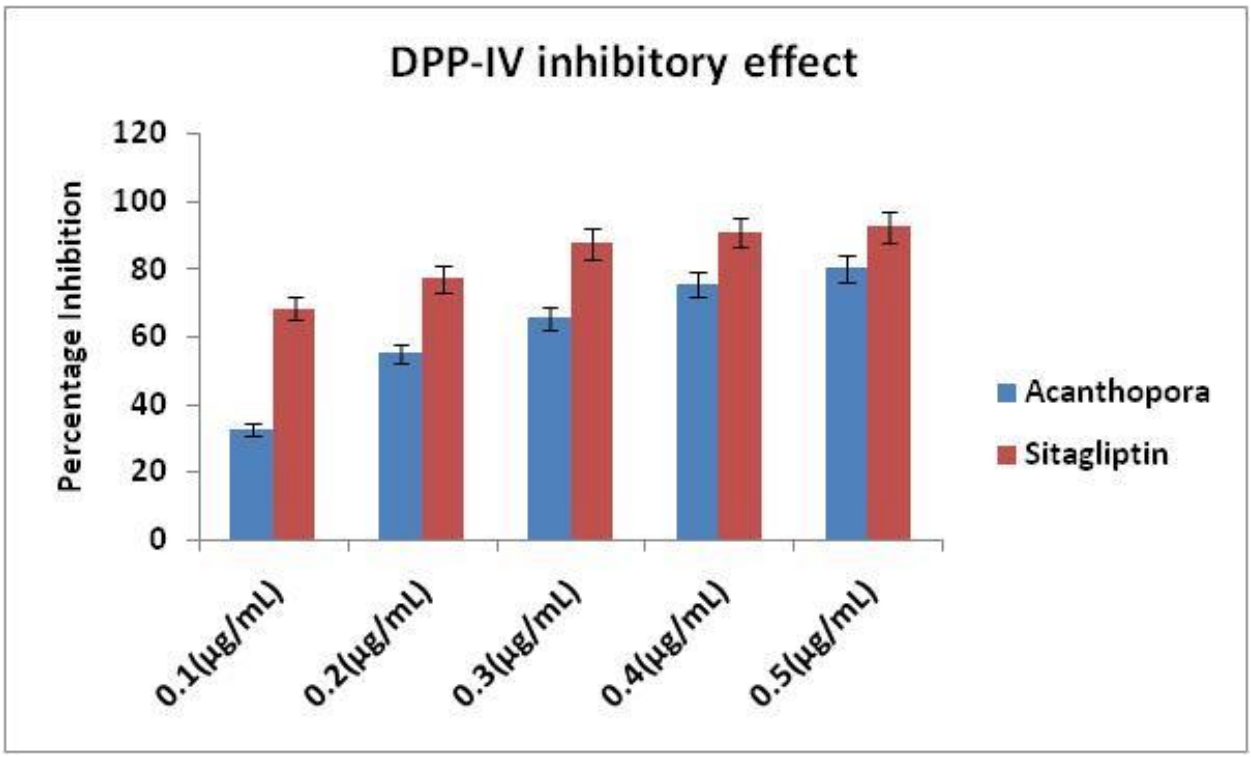

Table 2 DPP-IV inhibitory effect.

In this graph , we have taken x axis as concentration of the substance and y axis as percentage of inhibition of the substance. These are the main diabetic contributing agents .We have taken sitagliptin as standard and saw the effect of our substrate . The result shows that our extract shows minimum IC50 value of 0.4982µg/mL for DPP-IV.

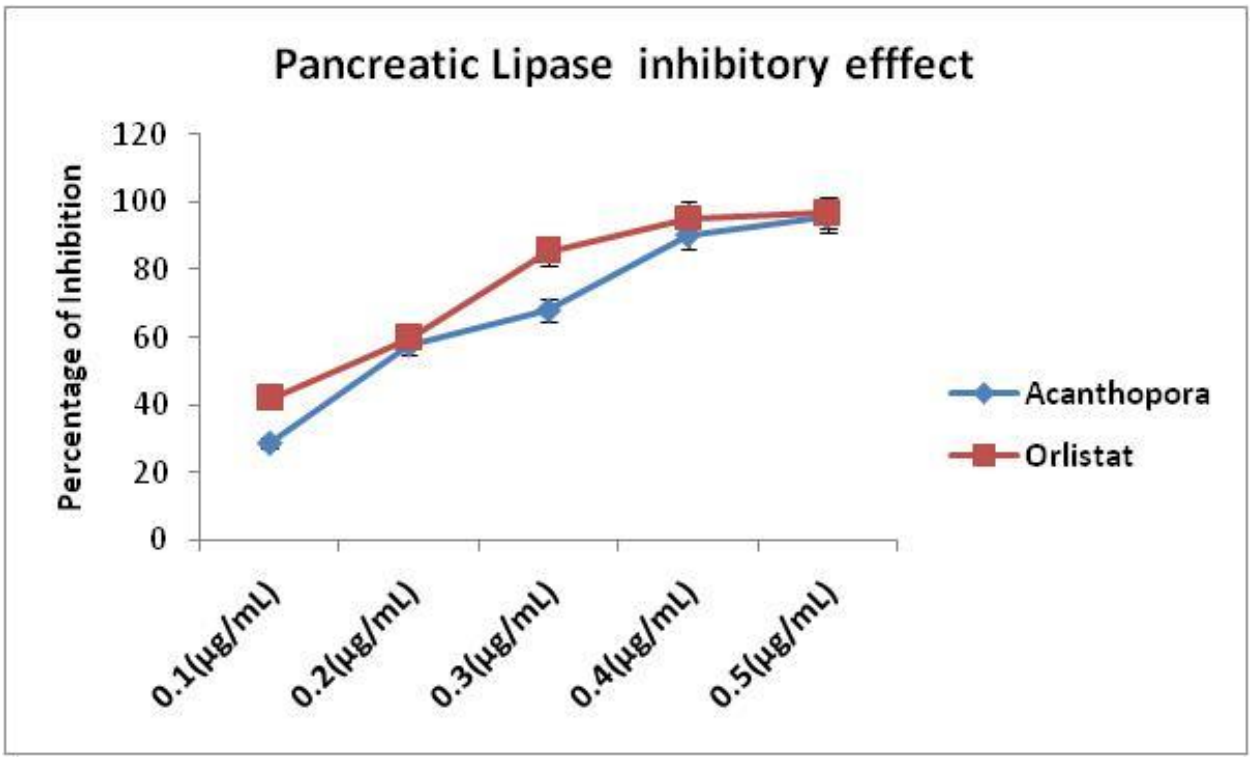

Table 3 Pancreatic lipase inhibitory effect .

In this graph , we have taken x axis as concentration of the substance and y axis as percentage of inhibition of the substance. These are the main diabetic contributing agents .We have taken orlistat as standard and saw the effect of our substrate . The result shows that our extract shows minimum IC50 value of 0.2µg/mL for pancreatic lipase inhibitory effect .

DISCUSSION

The polyphenols contained in this natural product are suspected to be responsible for some of its pharmacological effects. For example, Bu et al. reported that phenolic compound, butyl-isobutyl-phthalate isolated from brown seaweed, Laminaria japonica, had α-glucosidase inhibitory property. In the presnt study Acanthopora seaweed extract has a significant antidiabetic effect with lowering alpha glucosidase enzyme, DPP-IV and Pancreatic lipase. (12)

Recent research has focused on the in vitro antidiabetic effects of Acanthophora seaweed extract, and a number of related articles have examined the role that natural compounds may play in the management of diabetes. An examination of these studies' comparative analyses offers insightful information about the distinctive contributions and limitations of the results.(13)

Examining the antidiabetic effects of polysaccharides and polyphenols from diverse natural sources is a significant area of comparison. Numerous investigations have examined the potential antidiabetic effects of polysaccharides derived from various seaweed species, including

Sargassum sp. and Ascophyllum nodosum. These investigations have shown that these polysaccharides, like the seaweed extract Acanthophora, have inhibitory activity against α-amylase and α-glucosidase. But unlike other seaweed species, Acanthophora's polysaccharide composition and structure may be unique, which could have an impact on the bioactivity of the molecule. (14)

Acanthophora seaweed extract's polyphenolic content can be compared to extracts from other sources, like cinnamon, green tea, and blueberries, to show the variety and abundance of these bioactive substances in various natural products. Benefits specific to each source of polyphenols, such as anti-inflammatory and antioxidant properties, may apply to the treatment of diabetes and its aftereffects. (15,16)

Acanthophora seaweed extract has direct effects on oxidative stress and glucose metabolism; it may also have indirect effects on important enzymes involved in hepatic glucose synthesis and lipid metabolism. According to studies, the extract can inhibit the activity of the enzyme hepatic glucose-6-phosphatase, which lowers the production of endogenous glucose and helps to regulate blood glucose. Furthermore, Acanthophora seaweed extract may influence lipid metabolism and potentially improve diabetic dyslipidemia, a common complication in diabetes, by modulating lipogenic enzymes and lipoprotein lipase. (17)

It is important to recognize the difficulties and constraints in transferring the encouraging in vitro evidence of Acanthophora seaweed extract's antidiabetic effects to clinical applications. Although studies conducted in vitro yield important preliminary data, they are not able to accurately mimic the intricacies of the human body and how it reacts to various forms of treatment. Consequently, to confirm the safety and effectiveness of Acanthophora seaweed extract as a possible therapeutic agent for diabetes, preclinical research utilizing animal models and, eventually, carefully planned clinical trials involving human participants are essential. (18,19)

Regarding its anti-diabetic effects, Acanthophora seaweed extract's antioxidant qualities are also noteworthy. Oxidative stress, which is linked to diabetes, is defined as an imbalance between the body's antioxidant defense mechanisms and the production of reactive oxygen species (ROS). The development of diabetic complications, insulin resistance, and β-cell dysfunction can all be attributed to this oxidative stress. The high polyphenolic content of Acanthophora seaweed extract has the ability to neutralize oxidative damage and scavenge reactive oxygen species (ROS), which may lessen the impact of oxidative stress in diabetes. (20,21)

The mechanism by which Acanthophora seaweed extract enhances glucose metabolism is another topic of discussion. The antidiabetic effects of the extract have been explained by some

studies that have concentrated on particular pathways, such as GLUT4 translocation and hepatic enzyme modulation. Researchers can better grasp the possible targets and pathways involved in diabetes management by comparing these mechanisms with those found in other natural compounds. (22)

Moreover, reported results may differ depending on differences in experimental setups, such as the cell lines or assays used. Encouraging similar articles to use common model systems and standardize experimental protocols can improve the comparability and reliability of results.

As a future prospect isolating the compounds from Acanthopora seaweed and testing it individually against diabetic model both in-vitro and in-vivo will help to make antidiabetic drug development.

CONCLUSION

Preliminary experimental results demonstrate the significant antidiabetic efficacy of Acanthopora extract. By conducting in-vivo experiments, this research will be furthered and drug candidates for advanced biomedical research will be isolated.

The discussion of Acanthophora seaweed extract's in vitro antidiabetic effects highlights the plant's potential as a natural source of bioactive compounds for the treatment of diabetes. Similar articles can be compared to find similarities in the mechanisms of action of other natural products like polysaccharides and polyphenols. However, more research is necessary to determine the safety and efficacy of Acanthophora seaweed extract as a potential antidiabetic therapy due to its distinct composition and the variety of experimental setups. This research should involve preclinical and clinical studies. This subject adds to the increasing interest in using natural substances to manage diabetes, offering promising opportunities to create cutting-edge and potent strategies to reduce the prevalence of diabetes worldwide.

FUTURE SCOPE OF REFERENCE

Even though the in vitro results are encouraging, more investigation is necessary to confirm the extract's potential as a workable antidiabetic treatment, including preclinical and clinical studies. The combined results open up new avenues for successful diabetes management and add to the growing body of research on natural remedies as possible adjuncts or substitutes for conventional diabetes treatments.

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