Original Research Article

ANTI-DIABETIC & ANTIOXIDANT ACTIVITY OF PTEROCARPUS SANTALINUS AND STEVIA HERBAL FORMULATION

RUNNING TITLE: Antidiabetic and antioxidant activity of Pterocarpus santalinus and stevia.

Abstract

Introduction: *Pterocarpus santalinus* have their application in the pharmaceutical, cosmetic, agricultural, and food industries. *Stevia (Stevia rebaudiana)* is a natural, non-caloric sugar substitute that is a rich source of a pharmacologically significant glycoside. Proper diet, exercise, and pharmacological interventions contribute to overcoming diabetes.

Aim: The present study aims to assess the anti-diabetic and antioxidant activity of *Pterocarpus santalinus and Stevia* herbal formulation.

Materials and method: Preparation of plant extract followed by antidiabetic and antioxidant activity.

Results: Results were tabulated and graphically analyzed using SPSS software.As the concentration increased the percentage of inhibition also increased in both antidiabetic and antioxidant activity.

Conclusion: The present study concluded that *Pterocarpus santalinus and Stevia* herbal formulation has antidiabetic and antioxidant activity.

Keywords: *Pterocarpus santalinus, Stevia*, antidiabetic, antioxidant, Innovative method.

Introduction

Herbal drugs are commonly considered as less toxic and hence no side effects are associated (1). Diabetes is a growing health concern and an emerging global epidemic. It is a major challenge to control diabetes. Natural herbs have always been a source of drugs for humans since time immemorial. Traditional medicine in India has used these natural herbs to control diabetics. According to the World Health Organization (WHO), 90% of the total population in most of the developing countries use plants and their products as traditional medicine for primary health care. There are about 800 plants with anti-diabetic potential. Thus there is a growing demand for research on natural products with anti-diabetic properties. Numerous studies have defined the benefits of medicinal plants with anti-hyper-glycaemic effects in controlling diabetes mellitus (2–4). In the Past it is clearly shown that medicinal plants have been used in traditional healing globally for a long time to treat diabetes; this is because such herbal plants have hypoglycemic properties and other beneficial properties

*Pterocarpus santalinus:*The medicinal plants have their application in the pharmaceutical, cosmetic, agricultural, and food industries. The use of medicinal herbs for curing disease has been marked in the past Saga of all civilizations. Humans in the prehistoric era were not aware of the health hazards and their association with irrational therapy. With the commencement of research in medicine, it was concluded that plants contain active principles, which are responsible, for the curative action of the disease (5,6). Before the synthetic era, man was fully dependent on medicinal herbs for the prevention and treatment of various diseases. With the introduction of scientific procedures, medical practitioners were able to understand toxic principles present in the green flora. Medicinal plants serve as a natural source for most of the bioactive medicinal compounds (7,8). Medicinal plants like *Pterocarpus santalinus* are commonly used in traditional medicine which is rich in phenols and flavonoids. *Pterocarpus santalinus* shows antidiabetic activity and helps in decreasing the increased glucose levels and improving hyperlipidemia and restoring the insulin levels.(9).

Stevia (Stevia rebaudiana) is a natural, non-caloric sugar substitute that is a rich source of a pharmacologically significant glycoside. Proper diet, exercise, and pharmacological interventions contribute to overcoming diabetes. The pharmacological drugs indicated for treating diabetes are expensive and have certain adverse side effects(10). Therefore, herbs are considered a natural source of drugs that have strong antioxidant activities to be more effective against diabetes. Stevia rebaudiana Bertoni is a traditional plant that is famous because of its sweet taste and beneficial effects on blood glucose regulation. Stevia rebaudiana Bertoni (family Asteraceae) is popularly known as stevia, sweet weed, honey leaf, and the sweet herb of Paraguay (11). Stevia leaves contain a complex mixture of diterpene glycosides including stevioside, steviolbioside, rebaudiosides (A, B, C, D, E), and dulcoside A, the major sweet constituents are stevioside and rebaudioside A . Natural non-caloric sweetener stevioside (a major component of stevia) is considered to be sweeter than sucrose and is extensively used as a non-caloric sugar substitute in many application like foods, medicine, beverage, cosmetics, winemaking, household chemical industry, and other food industries. It cuddles anti-hyperglycaemic, anti-hypertensive, anti-oxidant, anti-tumor, anti-diarrheal, diuretic, gastro and renal-protective, and immunomodulatory properties (12). Herbal products like *Pterocarpus Santalinus* and *Stevia* are less toxic and have fewer side effects and so can be used daily. This study aims to assess the antidiabetic and antioxidant activity of *Pterocarpus santalinus* and *stevia* herbal formulation.

MATERIALS & METHODS:

Preparation of plant extract

Commercially available dry powder of *Pterocarpus santalinus* and *stevia* was readily available and was used for this experiment(Figure1). This experiment was conducted in Saveetha Dental College, Chennai, Tamilnadu. The experiment was carried out by dissolving 1g of Pterocarpus santalinus and stevia in 100 ml of water. The moisture was then boiled in a heating mantle at 70degrees Celsius for up to 10 minutes. The boiled mixture was then filtered using Whatman number 1 filter paper to obtain the plant extract. Then 40µl of the plant extract was measured using a measuring cylinder which might not be that accurate and the mixture was added to 60 ml of 1 mM dissolved in 60 ml of distilled water. (Figure 2)

Figure 1: Weighing 1g of *Pterocarpus santalinus* and *Stevia* herbal formulation.

Figure 2: Heating *Pterocarpus santalinus* and *Stevia* extract at 70°C for 10 minutes for the preparation of plant extract.

ANTIOXIDANT ACTIVITY:

1.DPPH METHOD(2,2-diphenyl-1-picrylhydrazyl)

Antioxidant activity

DPPH assay was used to test the antioxidant activity of *Pterocarpus santalinus* and *Stevia* herbal formulation. Diverse concentrations (2-10 µg/ml) of plant extract were mixed with 1 ml of 0.1 mM DPPH in methanol and 450 µl of 50 mM Tris HCl buffer (pH 7.4) and incubated for 30 minutes. Later, the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517 nm. BHT was employed as control. (Figure3) The percentage of inhibition was determined from the following equation,

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Figure 3:Antioxidant activity performed by adding 1ml of DPPH in methanol and then the reduction in the quantity of DPPH free radicals was assessed dependent on the absorbance at 517nm.

In-Vitro anti-diabetic assay

The in Vitro anti-diabetic assay was performed using two different techniques:

Alpha-Amylase inhibitory assay

Alpha-amylase inhibition was determined by quantifying the amount of maltose liberated during the experiment. The method reported by Bhutkar and Bhise has been followed(Bhutkar and Bhise,2012). Different concentrations of nanoparticles(20,40,60,80,100µl) was pre-incubated with 100% α amylase solution(1U/mL) at room temperature for 30 minutes. 100µl of starch solution(1%w/v) was further added to it and the mixture was incubated at room temperature for 10 minutes. 100µl of 96mM(3,5- dinitrosalicylic acid solution)DNSA reagent was added to it stop the reaction and the solution was heated in a water bath for 5 minutes. (Figure 4) Control was maintained where the equal quantity of enzyme extract was replaced by sodium phosphate buffer maintained at a pH value of 6.9. Reading was measured at 540nm. The experiment was performed in triplicate. Acarbose was used as a positive control.

The %inhibition was calculated using the formulae-

% inhibition =C-T/C*100

Where, C=control, T=test sample

Figure 4: Antidiabetic activity was performed by adding DNSA reagent to stop the reaction and the solution was heated in a water bath for 5 minutes.

Validation was done with nano experts followed by correlation analysis using SPSS software. Only antidiabetic and antioxidant activity was performed. In future studies, cytotoxicity and antimicrobial activity will be performed.

RESULTS:

ANTIOXIDANT ACTIVITY		
Concentration	**Percentage of inhibition**	**Wavelength**
10µl	**0.16**	**5.17nm**
20µl	**0.28**	**5.17nm**
30µl	**0.42**	**5.17nm**
40µl	**0.58**	**5.17nm**
50µl	**0.73**	**5.17nm**

Table 1: Antioxidant activity of *Pterocarpus santalinus* and *Stevia* herbal formulation.When the concentration was 10µl,20µl,30µl,40µl,50µl the percentage of inhibition was 0.16%,0.28%,0.42%,0.58%,0.73 respectively.

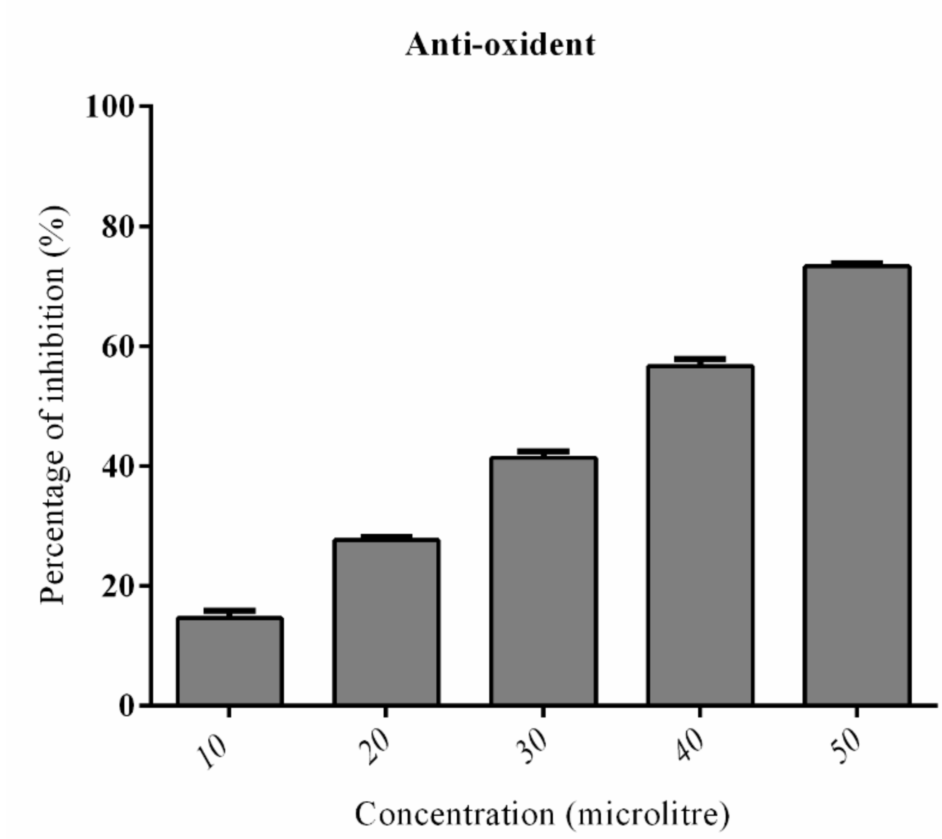

Figure 5:The graph represents the antioxidant activity of the extract. The X-axis represents different concentrations and Y-axis represents the antioxidant activity of the plant extract.When the concentration was 10µl,20µl,30µl,40µl,50µl the percentage of inhibition was 0.16%,0.28%,0.42%,0.58%,0.73 respectively.

ANTIDIABETIC ACTIVITY		
Concentration	**Percentage of inhibition**	**Wavelength**
10µl	**0.06**	**540nm**
20µl	**0.15**	**540nm**
30µl	**0.20**	**540nm**
40µl	**0.24**	**540nm**
50µl	**0.31**	**540nm**

Table 2: Antidiabetic activity of *Pterocarpus santalinus* and *Stevia* herbal formulation.When the concentration was 10µl,20µl,30µl,40µl,50µl the percentage of inhibition was 0.06%,0.15%,0.20%,0.24%,0.31 respectively.

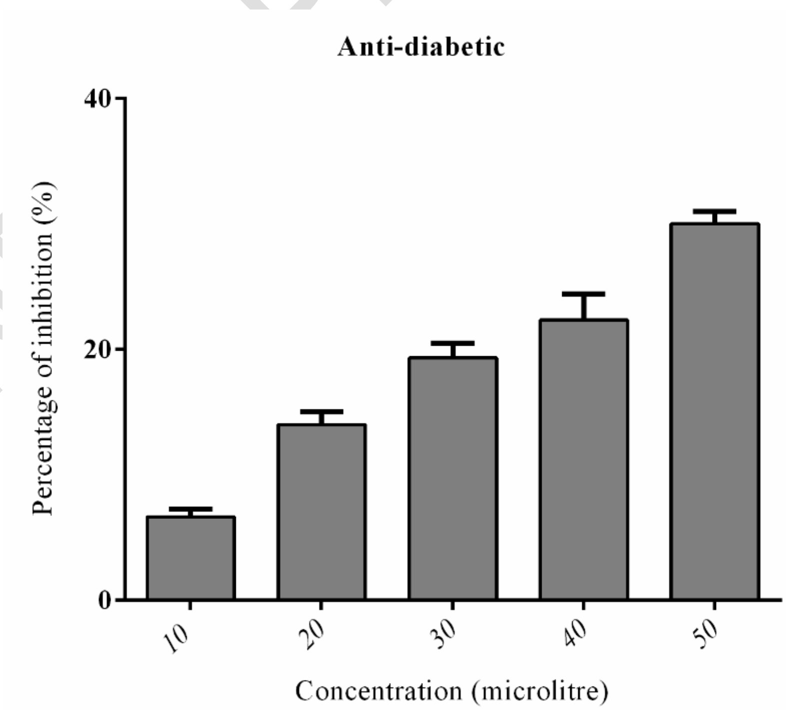

Figure 6:The graph represents the antidiabetic activity of the extract. The X-axis represents different concentrations and the Y-axis represents the antidiabetic activity of the plant extract.When the concentration was 10µl,20µl,30µl,40µl,50µl the percentage of inhibition was 0.06%,0.15%,0.20%,0.24%,0.31 respectively.

DISCUSSION:

In Table 1 & Figure 5 when the concentration was 10µl,20µl,30µl,40µl,50µl the percentage of inhibition was 0.16%,0.28%,0.42%,0.58%,0.73 respectively.We can see that antioxidant activity increased with an increase in concentration.Streptozotocin-induced Diabetes mellitus is exemplified not only by impaired glucose tolerance and hyperglycemia, as well by low antioxidant activity. The *Pterocarpus santalinus* has a strong antioxidant activity which has been demonstrated via different in vitro assays, as well as by using liver-slice slides (13,14). Even though the β-cell cytotoxic effect of Streptozotocin induction is not fully understood, it is considered to result in the inhibition of free radical scavenger enzyme production. Oxidative stress is held responsible for tissue damage and β-cell dysfunction. It is observed that an increase in HDL and reduction in LDL, has been observed in the combination with vitamin E and P. Body, muscle, heart, kidney, and liver weight are completely perceived to benefit from the combinational approach (15). Weight loss due to excessive breakdown of tissue proteins is also a complication of Diabetes Mellitus. Weight loss in treated groups either with *P. santalinus* or vitamin E alone or in combination was less significant, as compared to the untreated-diabetic group, which lost a considerable amount of weight. The pooled treatment with *Pterocarpus santalinus* and vitamin E exhibited promising results. an improvement in body weight, lipid profile, glucose tolerance, biochemical parameters such as urea, creatinine, and histological reversal of the nephropathy and activity of antioxidant enzymes were noticed. These results illustrated the anti-diabetic activity antioxidant action of *Pterocarpus santalinus.* The previous study was done by M. Eshrat Halim. stands in line with our present study stating the antidiabetic and antioxidant activity of *Pterocarpus santalinus.* (16)

Free radicals are electrically charged molecules, and a disproportionate generation of these free radicals is linked to many human diseases. Reactive oxygen species (ROS) along with hydroxyl radicals cause damage to the structure and function of cells, oxidation of lipids, proteins, and DNA; leads to the development of various diseases (17). The free radical scavenging activity of extracts of the leaves of *Pterocarpus santalinus* has been evaluated through in vitro studies. The methanolic extract of the leaves exhibited radical scavenging activity for diphenyl picrylhydrazyl (DPPH), nitric oxide, and hydrogen peroxide. Studies have proved that Fe3+ reducing capacity and DPPH radical scavenging activity in the methanolic extract of heartwood. As the concentration increased the antidiabetic and antioxidant activity also increased which was compared with butylated hydroxyanisole(BHA). Few other studies also showed the strong antioxidant activity against free radicals like DPPH,2,2'azinobis, hydroxyl, superoxide, and hydrogen peroxide. The present study is in line with the previous study done by Saradamma Bulle (18).

In Table 2 & Figure 6 when the concentration was 10µl,20µl,30µl,40µl,50µl the percentage of inhibition was 0.16%,0.28%,0.42%,0.58%,0.73 respectively.We can see that antidiabetic activity increased with an increase in concentration.*Stevia* leaf extract has been conventionally used to treat diabetes. Their ingestion causes a slender suppression of plasma glucose levels and significantly increases the glucose tolerance in human beings. Steviol glycosides have an enhancing effect on insulin secretion by directly acting on β-cells without altering the K+ - ATP channel activity and cAMP level in the islets, thus recording stevioside and steviol as potent antihyperglycemic agents. Stevioside regulates blood glucose levels by enhancing not only insulin secretion but also insulin utilization (18,19). Overall, *Stevia* possesses the ability to boost the insulin effect on cell membranes, increase insulin production, stabilize glucagon secretion and blood sugar levels, when carbohydrates are ingested improves glucose tolerance and lowers postprandial blood sugar levels in both animals as well as humans. Alternatively, it is described that *Stevia* is shown to provide a comprehensive set of mechanisms that counter the mechanics of type II diabetes and its eventual complications. Thus, to support healthy glucoregulation sugars can be replaced with steviol glycosides or stevioside of *Stevia* leaf. Adding to it not only the leaves of *Stevia*, dried or in powder form in supplementary food products of diabetic patients aid in increasing the natural sweetness and also help in rejuvenating the pancreatic gland (20).

The serum insulin level in the diabetic control group decreased due to Streptozotocin that resulted in diabetes by the rapid depletion of β-cells, which reduced the insulin release. An inadequate release of insulin causes hyperglycemia, which results in oxidative damage by the generation of reactive oxygen species and the development of diabetic complications (20,21). When *Stevia* aqueous extracts at different dose levels the insulin levels improve significantly due to the presence of natural components (stevioside) in *Stevia* leaves that are related to inhibition of hepatic expression of phosphoenolpyruvate carboxykinase and gluconeogenesis together with stimulation of hepatic glycogen synthesis that increased insulin secretion and insulin sensitivity. Evidence from other studies revealed that through the PPARγ-dependent mechanism *Stevia* aqueous extract elevates the insulin level due to stevioside which acts on pancreatic tissue, exerts beneficial anti-hyperglycemic effects. The study done by Uswa Ahmad states that *Stevia* aqueous extract enhanced caloric management and weight control by decreasing the feed intake and body weight gain, as per the present Study (13).

The Intent of an antidiabetic therapy in insulin-dependent patients (Type 1 diabetes) and insulin-non dependent patients (Type 2 diabetes) is to attain normoglycemia and reduce insulin resistance to improve metabolic control and prevent future diabetic patients (22). An effective strategy for the management of Type 2 diabetes is the inhibition of the two enzymes, alpha-amylase, and alpha-glucosidase, to slow the rate of absorption of carbohydrates, thus altering the postprandial rise of blood sugar and reducing the effects of diet on hyperglycemia. Undue inhibition of alpha-amylase due to alpha-glucosidase inhibitors such as acarbose carries certain side effects (bloating, flatulence, meteorism, and diarrhea) as a result of an abnormal bacterial fermentation of undigested carbohydrates in the colon (23,24).

According to a previous study, the alpha-amylase and alpha-glucosidase inhibitors derived from plants have mild inhibitory activity against alpha-amylase and strong inhibitory activity against alpha-glucosidase, which indicates their utilization in therapy for postprandial hyperglycemia with fewer side effects. This research reveals that the aqueous extracts of *Stevia* rebaudiana have significant inhibitory activity against the alpha-amylase and alpha-glucosidase enzymes and show capability for the treatment of both types of diabetes.Our team has extensive knowledge and research experience that has translate into high quality publications (23,25)(26,27)(28,29)(30,31)(32–34)(35–44). This activity can be performed with more concentration, the only anti-diabetic, and the antioxidant property is analyzed, clinical trials can be done. Because of its antioxidant property, it can be used as an anti-cancer drug. As it also shows anti-diabetic properties it can be standardized and given for patients with all types of diabetes.

CONCLUSION:

The present study suggests that aqueous extract from *Pterocarpus santalinus and Stevia* had shown better anti-diabetic and antioxidant activity by decreasing the random blood glucose level and fasting blood glucose and glycosylated (HbA1c) hemoglobin while insulin and liver glycogen levels significantly increased. It is implicit from the results that *Stevia* extract has anti-diabetic effects, and therefore could be used as a natural anti-diabetic drug for the treatment of diabetes and its associated complications.

NOTE:

The study highlights the efficacy of "Herbal" which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

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