

Herbal formulation of *Azadirachta indica* and *Stevia rebaudiana* and its anti-inflammatory and anti-diabetic activity.

ABSTRACT

INTRODUCTION:

Azadirachta indica, a natural herb also called *Neem*, and its seed extract has various medicinal uses. *Neem* also possesses notable insecticidal and pesticide activities. On the other hand, *Stevia rebaudiana* extract has alpha amylase glycosidase inhibitory activity, which contrasts with traditional wheat bread. This last plant can also regulate blood glucose levels, and prevent hypertension and caries.

AIM: This study aimed to analyse the anti-diabetic and anti-inflammatory activities of *Azadirachta indica* and *stevia rebaudiana*.

MATERIALS AND METHODS: 2.5 g from *Azadirachta indica* and *Stevia rebaudiana* plants mixed with 100 mL of distilled water were boiled. The final extract was then filtered using Whatman filter paper. Spearman correlation analysis was done by SPSS. Positive correlation analysis level of significance was set as $r=1$.

RESULTS: The anti-diabetic activity and anti-inflammatory activities of *Azadirachta indica* and *Stevia rebaudiana* extracts showed positive spearman correlations ($r = 1$). Both activities happen in a dose-dependent manner, being higher with the increasing of the concentration and of the absorbance values.

CONCLUSION:

Through this study, it was possible to conclude that *Azadirachta indica* and *Stevia rebaudiana* have great anti-inflammatory and anti-diabetic activities and can be used as a possible alternative drug for attenuating inflammation and preventing diabetic complications.

Keywords: *Stevia rebaudiana*, *Azadirachta indica*, Herbal formulation, Anti-inflammatory, Antidiabetic, Green synthesis.

INTRODUCTION

Azadirachta indica (*Neem*) is a natural herb also called Indian lilac or Neem tree. This plant and its seeds exhibit several medicinal uses. *Neem* also possesses insecticidal and pesticide activities. Additionally, its oil is a typical and effective pest repellent against sand fleas and mosquitoes. *Neem* can also control and repel moths, termites, ticks and fleas. They are also added to cattle grain to repel parasites and to control pest growth. *Neem* acts as a free radical neutralizer by enacting its active role as an anti-oxidant agent. *Neem* has also antimicrobial effects, being effective against several bacteria, viruses and fungi (1). Focusing on *Stevia rebaudiana*, this plant belongs to the Asteraceae (Sunflower) family of the genus *Stevia* (2). *Stevia* leaves contain alkaloids, flavonoids, chlorophylls, xanthophyll, aminoacid and lipids. It can grow to a height of 1–2 feet, and its leaves and flowers boost its flavour. *Stevia rebaudiana* belongs to a plant family of sweet herbs native to South America, Brazil, Southeast Asia and China. The plant extract has beneficial health properties, namely anti-hypertensive, anti-human rotavirus and anti-hyperglycaemia effects (3).

Diabetes mellitus is characterized by a rise in glucose level due to irregular metabolism of carbohydrates. 25% of the world population has diabetes, and its appearance happens in both developing and developed countries (4,5). Blood glucose levels are maintained by the human body within the constrictive range by glucagon and insulin. Type I diabetes is the inability to metabolize glucose with increased uptake by fat tissue and muscles (6). On the other hand, the causes of type II diabetes are mainly derived from environmental and dietary habits. Complications of diabetes involve hyperglycaemia, hyperosmolar state, diabetic neuropathy, hypoglycaemia, angiopathy and nephropathy (7). *Azadirachta indica* is reported to have medicinal values, including antidiabetic properties, as reported by Raajshree et al. using diabetic murine and rat models (8) Therefore, *A. indica* and *Stevia* is largely investigated owing to their abilities to control diabetes, scavenging free radicals and reactive species and anti-inflammatory effects. In fact, the antioxidant activity of *S. rebaudiana* extract already showed to be effective in a dose-dependent manner. The mode of anti-inflammatory activity of phenolic compounds is believed to be associated with their scavenging capacity through the donation of hydrogen atoms (9,10). *Stevia rebaudiana* reduces blood glucose, dental caries and prevents hypertension.

Likewise, it has shown effective anti-viral and anti-bacterial properties (11). The aim of the present investigation is the preparation of Herbal formulation of *A indica* and *S. rebaudiana* and to analyse its anti-oxidant and anti-diabetic activities (12-31)

Materials and methods:

Plant extract preparation:

Stevia rebaudiana and *Azadirachta indica* powdered extracts were collected from the herbal health care centre from where? (Figure 1) 100 mL of distilled water was added to 2.5 g of *A. indica* and *S. rebaudiana* in a beaker and boiled. The solutions were heated for 10-20 minutes in the heating mantle and cooled at room temperature (Figure 2). The boiled extract was filtered using Whatman filter paper. Random sampling method was done in an unbiased manner. Micropipetting had to be done with care to avoid manual error. The extract was prepared in Blue lab at Saveetha dental college and hospital, Chennai, country. The validation of the extract preparation procedure was done by Nano research experts.

Anti-diabetic activity:

Reagent and chemicals

- 3,5-dinitrosalicylic acid solution (DNSA) reagent (100 μ L)
- 5 mL of solutions of aqua alcoholic *B. diffusa* extracts of different concentrations (10 to 50 μ g/mL)

Positive control: acarbose

Negative control: α - amylase solution (100 μ L), starch solution (100 μ L) from which company?

ALPHA-AMYLASE INHIBITORY ASSAY:

Bhutkar and Bhise followed the quantifying method with liberated maltose amounts in the experiment to determine Alpha-amylase inhibition. 100µL of solution of alpha amylase (1U/mL) were pre-incubated with different concentrations (20µL, 40µL, 60µL, 80µL, 100µL) of the nanoparticles at 37 °C for 30 minutes (Figure 3). 100µL of starch solution was added (1% w/v) to the mixture at 37°C for 10 minutes. 3,5-dinitrosalicylic acid solution (DNSA) reagent 96 mM of 100 µL was added in order to stop further reaction of the solution and heated for 5 minutes in a water bath. Control and sodium phosphate buffer maintained at pH value of 6.9. Readings were recorded at 540nm. Acarbose was used as a positive control.

The formula used for the % inhibition was

$$\% \text{ inhibition} = \frac{C-T}{C} \times 100$$

Where, C= control, T= test sample

Anti-inflammatory activity:

Reagent and chemicals

- DPPH in methanol (1 ml of 0.1 mM) ,
- Herbal formulation of *Azadirachta indica* and *Stevia rebaudiana* (2-10 µg/ml)

Control group: Butylated hydroxytoluene

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

DPPH was held to test the antioxidant activity of *Azadirachta indica* and *Stevia rebaudiana* based herbal formulations. Diverse concentrations of extracts were added to DPPH at concentrations ranging from 1 mL to 0.1 mM in methanol. Therefore, 10 µl, 20 µl, 30 µl, 40 µl and 50µl of plant extract (2-10 µg/ml) were mixed with 1 mL of 0.1 mM DPPH in methanol and 450µl of Tris HCL buffer of 50mM (pH 7.4) were also added followed by 30 minutes incubation. The absorbances at 517nm were noted to observe the quantity of free radical reduction by DPPH. Butylated hydroxy toluene was the control. (Figure3). The percentage of inhibition was calculated by

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

The data obtained are analysed by spearman Rho correlation analysis using (IBM) SPSS 23 version. Positive correlation analysis level of significance was set as $r=1$.



Figure 1: Measured 2.5 g of *Neem* in a weighing machine.



Figure 2: Plant extract boiled at 60 °C for 10-20 minutes.

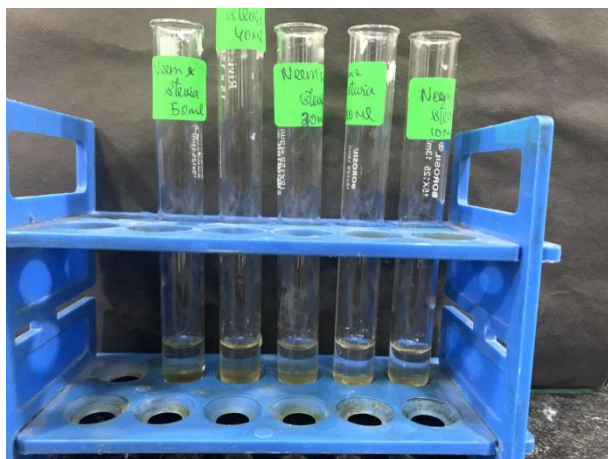


Figure 3: Incubation of nanoparticles with alpha amylase solution (100 μ L).

RESULTS

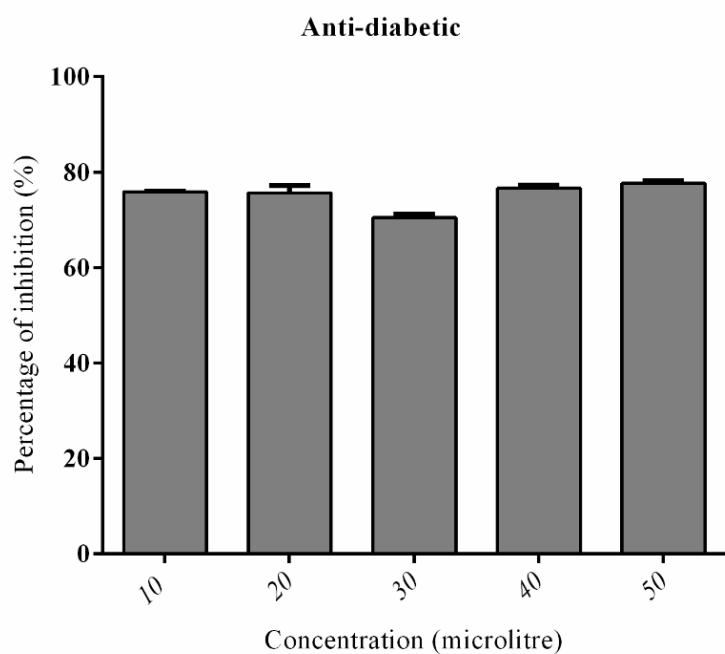


Figure 4: Anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* extracts was represented in the above graph. The X-axis represents the concentration of the extract in microlitre and the Y-axis represents the percentage of inhibition. Spearman positive correlation is seen between concentration (μ L) and antidiabetic activity, being higher with the increasing concentration (μ L)

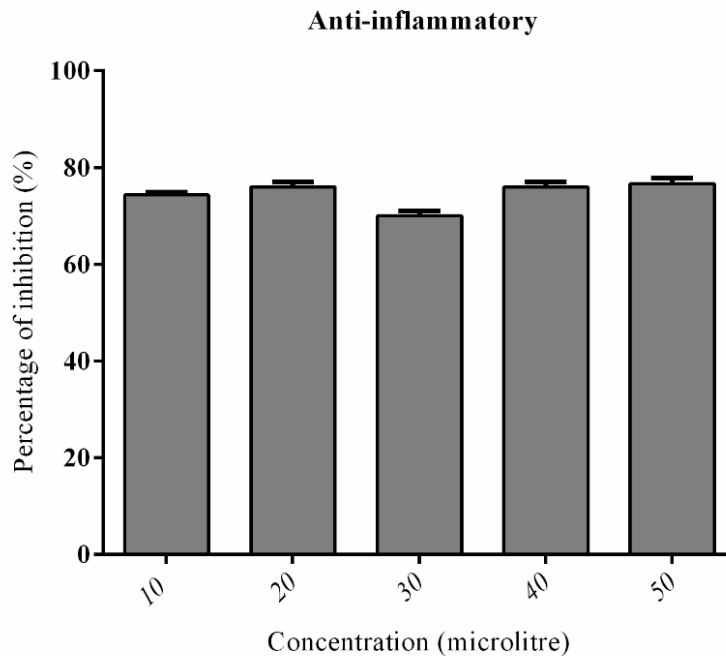


Figure 5: Anti-inflammatory activity of *Azadirachta indica* and *Stevia rebaudiana* extracts was represented in the above graph. The X-axis represents the concentration of the extract in microlitre and the Y-axis represents the absorbance. Spearman positive correlation ($r=1$) was observed with the increase of the concentration (μL).

The anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* extracts showed positive Spearman correlations ($r=1$) corresponding to the absorbance with the respective rise in the concentration of the extract (20 μL , 40 μL , 60 μL , 80 μL , 100 μL) (Figure 4). Anti-inflammatory activity of *Azadirachta indica* and *Stevia rebaudiana* showed positive correlations ($r=1$) with increased concentration followed by rising absorbance values and also shows statistical differences.

Graphs on antioxidant activity represented different concentrations (10 μl , 20 μl , 30 μl , 40 μl , 50 μL) in microlitre with absorbance of 0.75, 0.76, 0.71, 0.77, 0.78 at 660 nm. *Azadirachta indica* and *Stevia rebaudiana* extract showed positive anti diabetic activity ($r=1$) with increase in concentration with absorbance of 0.075, 0.076, 0.071, 0.077, 0.078 at 540 nm wavelength.

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DISCUSSION

This study revealed that *Neem* and *Stevia* together possess anti-inflammatory activity and anti-diabetic activities. It is clear that by increasing the concentration of *Azadirachta indica* and *Stevia rebaudiana* by adding 10 μ L every time, the percentage of inhibition on both anti-diabetic and anti-inflammatory activity increases. Other articles also based on the biological potential of *Azadirachta indica* and *Stevia rebaudiana* also show that both plants exhibit antioxidant, cytotoxic effects, anti-hyperlipidemic, anti-bacterial, anti-microbial and hepatoprotective effects (32). Correlation analysis was done to analyse anti-oxidant and anti-diabetic of *Azadirachta indica* and *Stevia rebaudiana* and WHAT?. This fact is not surprising and it is in accordance with the study of Satvika et.al.. These authors stated that *Neem* and *Aloe vera* synthesized silver nanoparticles have evident antidiabetic activity, namely by revealing notorious amylase inhibition (33). Furthermore, an in-vitro study regarding *Piperlongum* silver nanoparticles showed an effective anti-inflammatory activity with a maximum inhibition of 81.1% at 20 μ L concentration (34). Another studies showed that powdered seed of *Azadirachta indica* has good anti-diabetic activity (35,36). Previous studies done by Patil et al., already revealed that *Neem* and *Stevia* possess medicinal properties, like anti-inflammatory, anti-hypertensive and anti-oxidant activity (37) *Ginger oleoresin* combined with their silver nanoparticles have effective anti-oxidant and anti-inflammatory activities. Their activity increases in a dose-dependent manner (38). Additionally, anti-inflammatory activity was also tested in human blood samples with *Ocimum basilicum* and silver nanoparticles avenues. The obtained promising data can be also considered an added-value for this study. Selva priya et.al., have evaluated the anti-inflammatory activity and cytotoxic activity of *Neem* and *Stevia* herbal extracts. They obtained the highest absorbance at 50 μ L concentration with a maximum inhibition through albumin denaturation assay (39) Anti-inflammatory activity using silver nanoparticles and cumin oil can be used along with NSAIDS. Hypoinsulinemia and hyperglycaemia leads to diabetes mellitus development. The higher flavonoids content of plant extracts stimulates the beta cells of Langerhans to secrete insulin and reduce the glucose level, and in this way, restoring body weight. The attribution to hyperglycemia, hypoinsulinemia and increased proteolysis leads to the reduction in body weight after STZ-induced DM (40). The treatment of diabetic animals with the phenolic-rich plant extracts may result in improvements on body weight (41).

Previous study articles on alpha glucosidase and amylase inhibitory activity by *Stevia* plant extract showed inhibitory activity against alpha amylase and glucosidase in hyperglycaemia. (42–44). Some limitations of our study are the poor tested concentration and small sample number. Even so, works with increased concentration and a larger sample size can be done.

CONCLUSION

This study concludes that *Azadirachta indica* and *Stevi rebaudiana* have great anti-inflammatory and anti-diabetic activity and can be used as a possible alternative drug for attenuation, or even treating, inflammatory conditions and preventing diabetic complications. Even so, further research experiments have to be done to replace the adverse effects causing drugs with natural plant extract-based medications.

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