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

ABSTRACT

INTRODUCTION:

Azadirachta indica a natural herb and its seed extract has various medicinal uses. *Neem* is also believed for its insecticidal and pesticidal activities. *Stevia rebaudiana* extract has alpha amylase glycosidase inhibition which contrasts the traditional wheat bread. *Stevia rebaudiana* regulates blood glucose, prevents hypertension and caries.

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

MATERIALS AND METHODS: 2.5gms of *Azadirachta indica and Stevia rebaudiana* plant extract were added in 100 millilite of distilled water and boiled. The extract was filtered using whatmann 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 of *Azadirachta indica* and *Stevia rebaudiana* extract showed positive spearman correlation analysis (r=1) corresponding to the absorbance with respective rise in concentration of the extract. Anti-inflammatory activity and Anti-diabetic activity of *Stevia rebaudiana* and *Azadirachta indica* showed positive correlation with increased concentration followed by rise in absorbance at different concentrations.

CONCLUSION :

Through this study it was concluded that *Azadirachta indica and Stevia rebaudiana* have great anti-inflammatory and anti-diabetic activity and can be used as a possible alternative drug for treating inflammation and diabetic patients.

Keywords: *Stevia rebaudiana, Azadirachta indica,* herbal formulation, Anti Inflammatory, Antidiabetic, Green synthesis.

Running title: Anti-inflammatory and anti-diabetic activity of *Stevia rebaudiana* and *Azadirachta indica*

INTRODUCTION

Azadirachta indica (Neem) is a natural herb also called Indian lilac or Neem tree. *Azadirachta indica* a natural herb and its seed extract has various medicinal uses. *Neem* is also believed for its insecticidal and pesticidal activities. *Neem oil* was a typical and effective pest repellant, against sand fleas and mosquitoes. *Neem* control and repel moths, termites, ticks and fleas. They are also added to cattle grain to repel parasites and also helps in pest control. *Neem* acts as a free radical neutralizer by enacting its active role as an anti-oxidant. *Neem* has antimicrobial effects and can be effective against several bacteria, viruses and fungi (1). *Stevia rebaudiana* is a plant species belonging to the Asteraceae (Sunflower) family of the genus *Stevia* (2). *Stevia* leaves contain alkaloids, flavonoids, chlorophylls, xanthophyll, aminoacid and lipids. Stevia can grow to a height of 1–2 feet with leaves and flowers that boost the flavours of the leaves. *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 effects on anti-hypertensive, anti-human rotavirus and anti-hyperglycaemia(3).

Diabetes mellitus was characterized by a rise in glucose level due to irregular metabolism of carbohydrates. 25% of the world population were affected from diabetes in both developing and developed countries (4,5). Blood glucose level maintained by the human body within the constrictive range by glucagon and insulin. Type I diabetes is the inability to metabolize the glucose with increased uptake by fat tissue and muscles (6). Causes of Type II Diabetes are environmental and dietary habits. Complications in diabetes involve hyperglycaemia, hyperosmolar state, diabetic neuropathy, hypoglycaemia, angiopathy and nephropathy (7). *Azadirachta indica* is reported to have medicinal values including antidiabetic properties. Study by Raajshree et al., on in-vivo diabetic murine model against Streptozotocin induced diabetes in albino rats (8) *A. indica and Stevia* is investigated for the biochemical parameters important for controlling diabetes, source of free scavenging activity and antiinflammatory activity. The antioxidant activity of *S.rebaudiana* extract was effective with a rise in concentrations. The

mode of anti-inflammatory activity of the phenolic component is believed to show an effective radical scavenging activity through donation of hydrogen atoms (9,10). *Stevia rebaudiana* reduces blood glucose, dental caries and prevents hypertension. Likewise it has shown an effective anti-viral and anti-bacterial property (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 activity.(12).(13–26) ,(27–31)

Materials and methods:

Plant extract preparation:

Stevia rebaudiana and Azadirachta indica powdered extract was easily available and collected from the herbal health care centre. (Figure 1) 100 ml of distilled water was added to 2.5gms of *A.indica and Stevia rebaudiana* in a beaker and boiled. The solutions were heated for 10-20 mins in the heating mantle and cooled at room temperature.(Figure 2) The boiled extract was filtered using whatmann filter paper. Random sampling method was done in an unbiased manner. Micropipetting has to be done with care to avoid manual error. The extract was prepared in Blue lab at Saveetha dental college and hospital, Chennai. 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 µL)
- 5 mL of solutions of aqua alcoholic *B. diffusa* extracts of different concentrations (10 to 50 µg/mL)

Positive control: acarbose

Negative control: α- amylase solution(100 µL), starch solution (100 µL)

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) are pre-incubated with different concentrations (20µL, 40µL, 60µL, 80µL, 100µL) of the nanoparticles at 37 degree celsius 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 millimole 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 maintained as a positive control.

Formula for the % inhibition was calculated using

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

Where, C= control, T= test sample

Antiinflammatory 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 were held to test the antioxidant activity of *Azadirachta indica* and *Stevia rebaudiana* based herbal formulations. Diverse concentrations of extracts were added to DPPH of 1ml of 0.1mM in methanol and 10µl,20µl,30µl,40µl,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 absorbance 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.

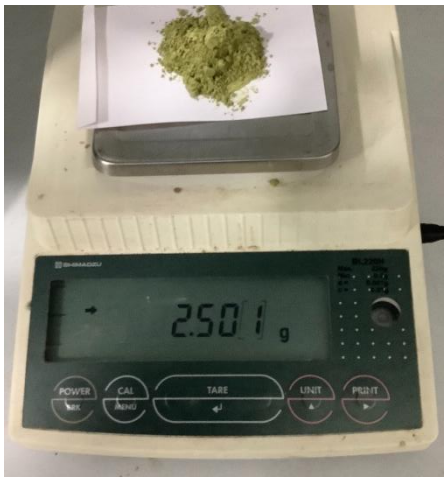

Figure 1: Measured 2.5gm of *Neem* in a weighing machine.

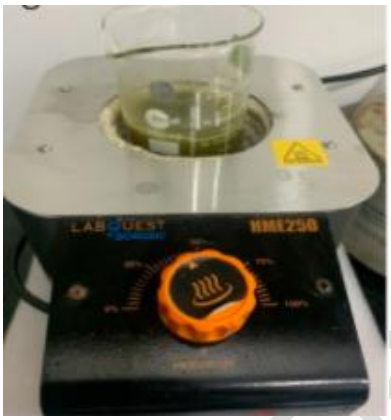

Figure2: Plant extract boiled at 60 degree celsius for 10-20 minutes

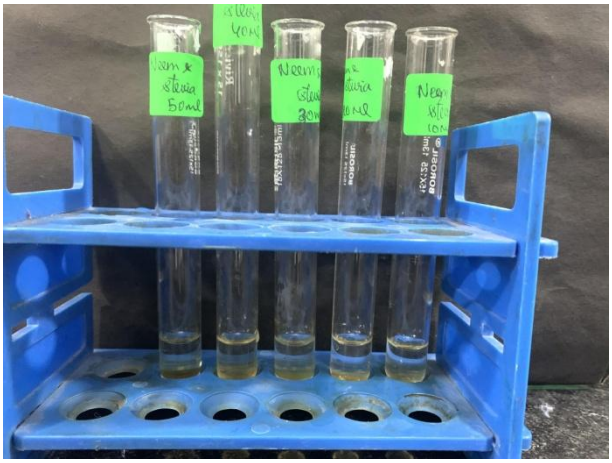

Figure 3: Incubation of nanoparticles with alpha amylase solution (100 µl)

RESULTS

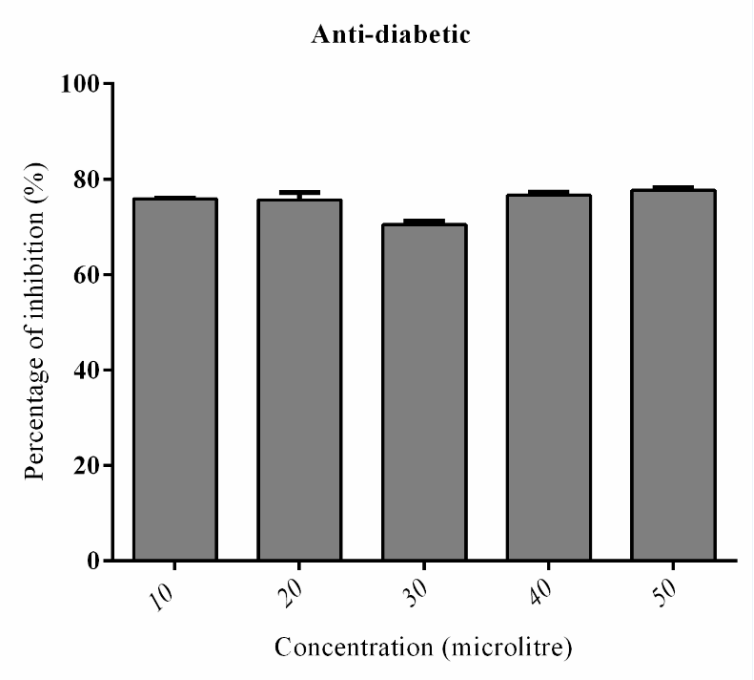

Figure 4: Anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* extract were represented in the above graph. The X-axis represents the concentration of the extract in microlitre and the Y axis represents the percentage of inhibition. Spearman positive correlation is seen between concentration (µl) and antidiabetic activity with increase in concentration(µl)

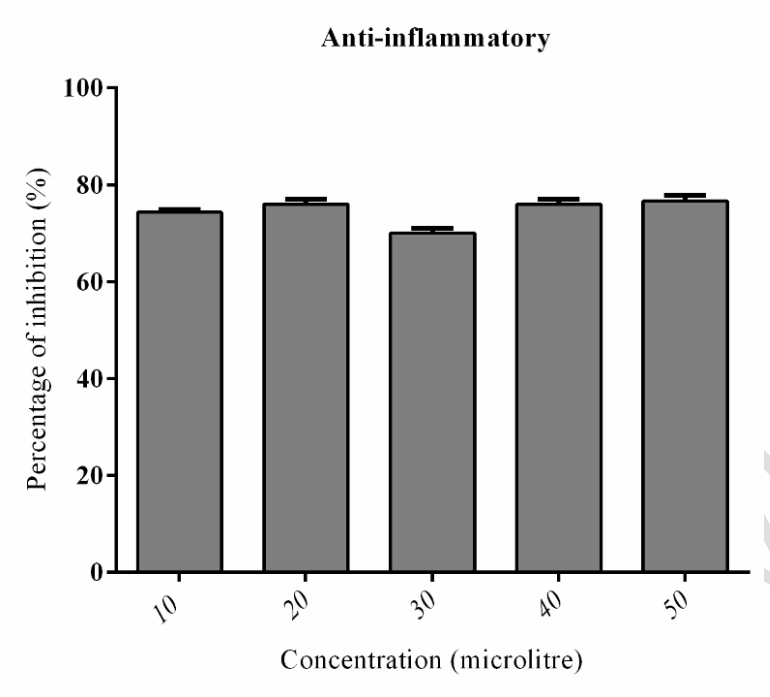

Figure 5: Anti-inflammatory activity of *Azadirachta indica* and *Stevia rebaudiana* extract were represented in the above graph. The X-axis represents the concentration of the extract in microlitre and the Y axis represents the absorbance. Spearmen positive correlation (r=1) was observed with increase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

The anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* extract showed positive spearman correlation analysis (r=1) corresponding to the absorbance with respective rise in concentration of the extract (20µL, 40µL, 60µL, 80µL, 100µL) (figure 4). Anti-inflammatory activity of *Azadirachta indica* and *Stevia rebaudiana* showed positive correlation (r=1) with increased concentration followed by rise in absorbance which is statistically significant.

Graphs on antioxidant activity represented different concentrations (10µl,20µl,30µl,40µl,50µl) in microlitre with absorbance of 0.75, 0.76, 0.71, 0.77, 0.78 at 660nm. *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 540nm wavelength.

DISCUSSION

The study revealed that *Neem and Stevia* together possess anti-inflammatory activity and anti-diabetic activity. It is clear that by increasing the concentration of *Azadirachta indica and Stevia rebaudiana* by adding 10µL every time it increases the percentage of inhibition on both anti-diabetic and anti-inflammatory activity. Other articles on *Azadirachta indica and Stevia rebaudiana* show that it has 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*. Study by satvika et.al., stated that *Neem* and *Aloe vera* synthesized silver nanoparticles have evident antidiabetic activity. It revealed good amylase inhibition (33). Invitro study on *Piperlongum* based Silver nanoparticles showed an effective anti-inflammatory activity with a maximum inhibition of 81.1% at 20 microlitre concentration (34). Another study which explains the effect of powdered seed of *Azadirachta indica* which has good anti-diabetic activity (35,36). Previous studies done by Patil et al., on *Neem and Stevia* revealed medicinal properties like anti-inflammatory, anti-hypertensive and anti-oxidant activity (37) *Ginger oleoresin* mediated silver nanoparticles have effective anti-oxidant and anti-inflammatory activity is detected in combination of *Ginger oleoresin* and silver nanoparticle extract. The activity increases by raising the concentration (38). Anti-inflammatory activity was analysed and tested against human blood with *Ocimum basilicum* and silver nanoparticles avenues against human bodily ailments which add on to its medicinal advantage. Selva priya et.al., have experimented the Anti-inflammatory activity and cytotoxic activity of *Neem and Stevia* based herbal extract which revealed highest absorbance at 50 microlitre concentration with a maximum inhibition through albumin denaturation assay (39) Anti-inflammatory activity using silver nanoparticles and cumin oil which can be used along with NSAIDS. Hypoinsulinemia and hyperglycaemia leads to diabetes mellitus. The high flavonoids content of plant extract stimulate the beta cells of langerhans to secrete insulin and reduce the glucose level restoring the body weight. The attribution to hyperglycemia, hypoinsulinemia and increased proteolysis leads to the reduction in body weight after STZ-induced DM (40). The treatment for the diabetic animals with the plant extract may result in the improvement of the body weight because of its high content of flavonoids (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). The limitations of our study on anti-inflammatory and anti-diabetic activity of *Azadirachta indica* and *Stevia rebaudiana* is its less concentration and small sample size. Studies with increased concentration and larger sample size can be done. Only anti-diabetic, and the anti-inflammatory property is analyzed and standardized form of anti-diabetic drug extract of *Azadirachta indica* and *Stevia rebaudiana* can be given for patients with diabetes after clinical trials in future.

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 treating inflammatory conditions and diabetic patients. Further research experiments have to be done to replace the adverse effects causing drugs with natural plant extract based medications.

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CONFLICT OF INTEREST:

The authors would like to declare no conflict of interest in the present study.

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COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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