An in vitro study to compare and evaluate the anti-diabetic effect of Tri-herbal leaf extracts nanoemulsion with Acarbose

Sushama Das¹, Karpagavel¹*, K.Murugavel¹, N.Manikandan¹

1. Department of Biochemistry, Chettinad Hospital & Research Institute (CHRI), Chettinad Academy of Research and Education (CARE), Rajiv Gandhi Salai, Kelambakkam- 603103, Tamilnadu, India

*Corresponding author
Dr. L. Karpagavel,
Professor,
Department of Biochemistry,
Chettinad Hospital & Research Institute (CHRI),
Chettinad Academy of Research and Education (CARE),
Rajiv Gandhi Salai, Kelambakkam- 603103, Tamil Nadu, India.
E-mail ID: karparg@yahoo.com

ABSTRACT

Introduction: Diabetes has become a global health concern, its management lies hugely on expensive medical care and monitoring; thus the need to investigate possible alternatives to reduce treatment cost and also to reduce the side effects of commonly used anti diabetic drugs. Methods: This study aims to assess the anti-diabetic effect of Tri-herbal leaf extract nanoemulsion of Murraya koenigii, Moringa oleifera and Coccinia grandis. Tri- herbal leaf extract nanoemulsion were prepared in the ratio of VCO oil:water:surfactant- 32:36:32 (%w/w). Characterization parameters of Tri- herbal leaf extract nanoemulsion including pH, zeta potential, size, morphology were within the standard limits and was used for the study. The anti diabetic effect of nano emulsified Tri- herbal leaf extract was compared with that of acarbose by measuring their inhibitory effect on the enzyme alpha glucosidase. Result: Maximum Inhibitory effect of formulated Nano emulsion on enzyme alpha glucosidase was 96% at concentration 81μg/ml whereas that for the standard anti-diabetic drug Acarbose was 93% at concentration 540μg/ml. For acarbose, the maximum Inhibition was 92% at the concentration of 540μg/ml. Conclusion: The relative inhibition Percentage of Nano emulsion versus Control has been analyzed and the results found to be statistically significant (P<0.003).

Keywords: Alpha glucosidase, Anti-diabetic, Tri-herbal leaf extract, Nanoemulsion


INTRODUCTION: Diabetes mellitus still continuous to be one of the leading cause of mortality and morbidity and it is estimated that by the year 2030, diabetes mellitus may affect up to 79.4 million individuals in India(Shrivastava, Shrivastava and Ramasamy, 2013).Although currently various classes of anti-diabetic drugs like insulin, sulfonylureas, biguanides, and glinides are available, numerous studies have reported these drugs to have a number of undesirable effects(Patel et al., 2012). So diabetic patients are still in need of natural preparation which may be better to comply with and have equal efficacy to oral anti diabetic drug. Many research article & review article have highlighted the anti diabetic effect of fruit juice extracts(Salehi et al., 2019).The purpose of the present in-vitro study is to evaluate the anti diabetic effect of Tri-herbal leaf extract nanoemulsion with known oral anti
diabetic drug like Acarbose. The anti-diabetic effect of nano emulsified Tri-herbal leaf extract was compared with that of acarbose by measuring their inhibitory effect on the enzymes like alpha glucosidase. Until now, based on the google search no such study has been done on the Nano emulsion formulation of Tri-herbal leaf extract of Murraya koenigii, Moringa oleifera and Coccinia grandis.

**MATERIALS AND METHODS:**

**Consumables and reagents:** Alpha-glucosidase, Para-nitro phenyl glyco pyranoside (PNPG), Acarbose, Sodium Carbonate, Sodium chloride, were purchased from Sisco Research Laboratories (SRL), Chennai.

**Glassware and apparatus:** ELISA reader (Bio-Rad PR4100) and UV double beam spectrophotometer (Shimadzu) were used to measure the absorbance.

The study was conducted in the Department of Bio-chemistry, Chettinad Academy of Research and Education (CARE), Chennai.

**Evaluation of the antidiabetic effect of mixed fruit juice nanoemulsion**

1. **α-glucosidase inhibition assay**

**Reagent**

1. 100mM phosphate buffer (pH 6.9)

0.819 g of Na₂HPO₄, 0.507 g of NaH₂PO₄ and 35 mg of NaCl were weighed and dissolved in 100 ml of Milli-Q-water

2. Alpha Glucosidase solution

Commercially available alpha glucosidase was (169 U/mg). 0.1 mg of Alpha Glucosidase was weighed and dissolved in 10 ml of 100 mM of Phosphate Buffer of pH 6.9 to obtain the concentration of 1.69 U/ml.

3. 1mM Para Nitro phenylα-D-Glucopyranoside (PNPG)

30.1 mg of PNPG was weighed and dissolved in 100 ml Milli-Q-water

4. 0.1M Sodium Carbonate

1.05 g of Sodium Carbonate was weighed and dissolved in 100 ml of Milli-Q-water

**α-glucosidase inhibition assay**

α-Glucosidase inhibition was done using modified methods described by Bachhawat et al and Mayur et al. Approximately, 10 μL α-glucosidase (1.69 U/mL), 50 μL sodium phosphate buffer (0.1 M, pH 6.9), six different concentrations of Nano emulsion sample were taken in different volumes 15 μl, 30 μl, 45 μl, 60 μl, 75 μl and 90 μl at concentration ranging from 13.5 μg to 81 μg and then they were incubated for 10 minutes in 37°C (fig 1). The total volume of test has been made up to 90 μl by using Milli-Q-water. Different concentrations of test were named as T1, T2, T3, T4, T5, and T6, 20 μL p-nitro phenol-α-D-glucopyranoside (PNPG) substrate (1 mM) was incubated at 37°C for 30 min. After incubation, 50 μl of sodium carbonate (0.1 M) was added to the reaction mixture to terminate the reaction. The hydrolysis of PNPG to p-nitro phenol was monitored using an UV visible double beam spectrophotometer at 405 nm. Positive Control Acarbose were also used in different volumes same like test 15 μl, 30 μl, 45 μl, 60 μl, 75 μl and 90 μl at concentration ranging from 90 μg to 540 μg, final volume of control has been made up to 90 μl with Milli-Q-water. The test samples were loaded in the wells of the micro titer plate in the order H1, H2, H3, H4, H5 and H6, Blank was loaded in G1 and the controls were loaded in the row of F1, F2, F3, F4, F5 and F6 in micro plate. The absorbances were read at 405 nm in UV visible double beam spectrophotometer. The percentage of inhibition were calculated by using the following formula.
RESULTS AND DISCUSSION

I α-glucosidase inhibition assay

Alpha glucosidase inhibitory effect of Tri-herbal leaf extract nanoemulsion and acarbose was assessed by the release of p-nitro phenol from PNPG. The IC$_{50}$ values of the formulated Tri-herbal leaf extracts (32μg/ml) on enzyme inhibition activity were found to be decreased when compared to positive control acarbose (265μg/ml). The maximum inhibition of the formulated nanoemulsion on alpha glucosidase enzyme activity was 96% at 81μg/ml. For the positive control acarbose the maximum inhibition was 92% at 540μg/ml (fig-2)

Statistical analysis: Data of the present research were expressed as Mean ± Standard Deviation. Statistical difference between the test samples and controls were measured with unpaired independent sample t-Test. Statistical one way ANOVA analysis of the data was performed by IBM SPSS-21 software with P value of <0.05 were considered statistically significant. The P values of the α-glucosidase inhibition assay are given below.

DISCUSSION: Drugs commonly used in the treatment of diabetes mellitus are Metformin and Acarbose (Rojas and Gomes, 2013). In spite of it, many research articles have reported, that these drugs have certain side effects. Gastro intestinal complications like flatulence and diarrhea are the commonplace side effect of acarbose & Metformin that have been reported (Fatima, Sadeeqa and Nazir, 2018). In the present health care system, Natural compounds of plant source are thus being preferred over synthetic drugs. Several fruit juice has been investigated for their anti-diabetic properties and is presently being used in Ayurveda (Nayak and De, 2013). The main disadvantage of the raw Leaf extract was during formulation and storage of the phyto constituents present in the raw fruit extract that are exposed to oxidation, hydrolysis, microbial attack and some other environmental degradation which leads to instability of the product (Thakur et al., 2011). Hence to overcome this above issue, the raw Tri-herbal leaf extracts of Nanoemulsion form were used. The present study was designed to investigate the potential effects of traditional medicinal plant leaves to inhibit the enzymes involved in hydrolyzing carbohydrates like α-glucosidase. The α-glucosidase inhibitors have been useful as oral hypoglycemic drugs for the control of hyperglycemia especially in patients with type II diabetes mellitus (‘Alpha Glucosidase Inhibitors - StatPearls - NCBI Bookshelf’). Nano formulations have many advantages in herbal drug formulation, when compared to traditional formulation, including enhancement of solubility, bio-availability, stability and pharmacological activity. In our present study, Nano emulsions of Tri-herbal leaf extract prepared in the ratio of 32:36:32 (virgin coconut oil, mixed fruit juice extract and tween-20) was used. Based on the review of literature on synthesis and characterization of Nano emulsions, the standardized ratio were used in this present study (Saxena et al., 2018). In this current research, Tri-herbal leaf extract Nano emulsion formulation has been designed to investigate the Anti-diabetic activities of Nano formulation at different concentrations and it has been compared with the standard drug acarbose. The various Inhibitory effects of synthesized Nano emulsion on alpha glucosidase activity were studied. The Data summarized reveals that the maximum Inhibitory effect of formulated Nano emulsion on enzyme alpha glucosidase was 96% at concentration 81μg/ml whereas the standard anti-diabetic drug Acarbose has 92% at concentration 540μg/ml. The study results validate that, the Tri-herbal leaf extract Nanoemulsion has significant inhibitory action on alpha glucosidase at lower concentration in contrast to standard anti diabetic drug Acarbose. Various concentrations of Nanoemulsion has been analyzed and compared with standard drug Acarbose and found to be statistically significant (P<0.003). The study data also proves that an increase in dose dependent inhibitory action on alpha glucosidase. The IC₅₀ values of Nanoemulsion and acarbose has been analyzed and it has been found to be highly significant (P<0.001). The Inhibition percentage of Nano emulsion varied from 24% to 96% at concentration ranging from 13.5μg to 81μg/ml. The obtained results also suggest that the Nano emulsion formulation of Tri-herbal leaf extract has high inhibitory effect on alpha glucosidase at low concentration. Inhibitory activity of plant phyto constituents on enzymes were highlighted by numerous scientific reports (Salehi et al., 2019). The Tri-herbal leaf extract nanoemulsion formulation combines the beneficial effects of all the three leaves such as Murraya koenigii,
Moringa oleifera and Coccinia grandis. It exhibits anti diabetic effect by scavenging free radicals, Inhibition of carbohydrate hydrolyzing enzymes, stimulating insulin secretion and regenerating β-cell architecture.

**CONCLUSION:** The present study has been designed based on the concept of famous quotes of Hippocrates, “*Let Food be thy Medicine and Medicine be thy Food*”. The in-vitro study results on evaluation of Anti-diabetic effect of Nano formulation clearly shows anti diabetic activity by inhibiting enzymes alpha glucosidase. Beneficial effects of three leaves such as Murraya koenigii, Moringa oleifera and Coccinia grandis Nano formulation enhances absorption, bio- availability, and stability and Pharmacological activity when compared to traditional phyto formulation. Side effects of Allopathic drugs reported in various journals can be overcome by this natural Nanoemulsion formulation. Inclusion of this natural Tri-herbal leaf extract Nano emulsion in our diet plan can definitely be useful in the treatment of Diabetes Mellitus. Further, studies are needed to be carried out in Animal model to support the evidence of this In-vitro study. This study paves a way for further screening of phyto constituents, which has potential Anti-diabetic activity and also toxicity of the Nanoemulsion formulation needs to be evaluated.

**REFERENCES**

1. ‘Alpha Glucosidase Inhibitors - StatPearls - NCBI Bookshelf’.