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An in-vitro study to compare and evaluate the anti-diabetic effect of mixed fruit juice nanoemulsion with acarbose

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ABSTRACT

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. This study aims to assess the anti-diabetic effect of mixed fruit juice nanoemulsion of Coccinia grandis, Punica granatum and Phyllanthus emblica. Mixed fruit juice nanoemulsion were prepared in the ratio of VCO oil:water:surfactant- 32:36:32 (%w/w). characterization parameters of mixed fruit juice nanoemulsion including P^H, zeta potential, size, morphology were within the standard limits and was used for the study. The anti diabetic effect of nano emulsified mixed fruit juice was compared with that of acarbose by measuring their inhibitory effect on the enzyme alpha glucosidase. Maximum Inhibitory effect of formulated Nano emulsion on enzyme alpha glucosidase was 83% 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. The relative inhibition Percentage of Nano emulsion versus Control has been analyzed and the results found to be statistically significant (P<0.003).

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INTRODUCTION

Diabetes mellitus still continuous to be one of the leading cause of mortality and morbidity (Li *et al.*, 2019). It is estimated that by the year 2030, diabetes mellitus may affect up to 79.4 million individuals in India (Shrivastava *et al.*, 2013).

The pathologic hallmark of DM involves the vasculature leading to both microvascular and macrovascular complications (Orasanu and Plutzky, 2010). Chronicity of hyperglycemia is associated with long-term damage and failure of various organ systems mainly affecting the eyes, nerves, kidneys, and the heart(Volume, 2016). Haemoglobin A1c (HbA1c) can be used both for monitoring and diagnosis (George and Erasmus, 2018). Achieving near-normal glycated hemoglobin significantly, decreases risk of macrovascular and microvascular complications (Marín-Peñalver *et al.*, 2016). Currently various classes of anti-diabetic drugs like insulin ,sulfonylureas, biguanides, and glinides are available (Sola *et al.*, 2015).Numerous studies have reported anti-diabetic drugs 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 (Dwivedi and Das-

paul, 2013). 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 mixed fruit juice nanoemulsion with known oral anti diabetic drug like Acarbose. Until now, based on the google search no such study has been done on the Nano emulsion formulation of fruit juice extract of Phyllanthus emblica, Coccinia grandis and Punica granatum (Nayak and De, 2013). Therefore this is the first study designed in Nanoemulsion form.

MATERIALS AND METHODS

Consumables and reagents

Alpha-glucosidase, Para-nitro phenyl gluco 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.

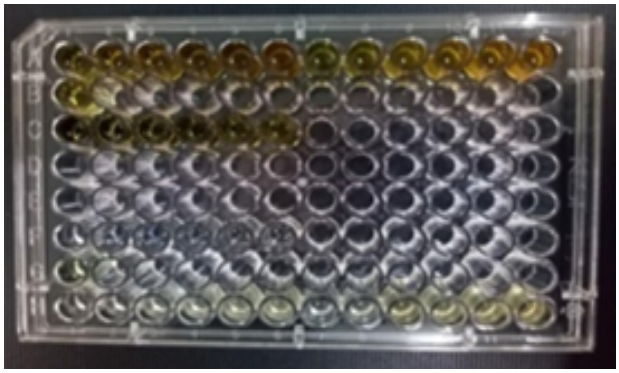

Figure 1: Microtitre plate containing Alpha glucosidase inhibition assay reaction mixture

Evaluvation of the antidiabetic effect of mixed fruit juice nanoemulsion

I . α-glucosidase inhibition assay

Reagent

1. 100mM phosphate buffer (pH-6.9)

0.819g of Na_2HPO_4, 0.507g of NaH_2PO_4 and 35mg of NaCl were weighed and dissolved in 100ml of Milli-Q-water

2. Alpha Glucosidase solution

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

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

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

4. **0.1M Sodium Carbonate**

1.05g 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)(Picot, Subratty and Mahomoodally, 2014), 50 μL sodium phosphate buffer (0.1 M, pH 6.9), six different concentrations of Nano emulsion sample were taken in different volumes 15μl, 30μl, 45μl, 60μl,75μl and 90 μl at concentration ranging from 13.5μg to 81μg and then they were incubated for 10minutes in 370C. The total volume of test has been made up to 90μl by using Milli-Q-waterFigure 1 . Different concentrations of test were named as T1, T2, T3, T4, T5, and T6. 20μL p-nitro phenol-α-D-glucopyranoside (PNPG) substrate (1mM) was incubated at 370C for 30 min. After incubation, 50 μl of sodium carbonate (0.1M) 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 15μl, 30μl, 45μl, 60μl,75μl and 90 μl at concentration ranging from 90μg to 540 μg, final volume of control has been made up to 90μl with Milli-Q-waterFigure 2 . 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 405nm in UV visible double beam spectrophotometer. The percentage of inhibition were calculated by using the following formula,

$$Inhibition\ \% = \frac{Abs_{\ control} - Abs_{\ sample}}{Abs\ control} \times 100$$

The IC_{50} values were determined from plots of percentage inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values.

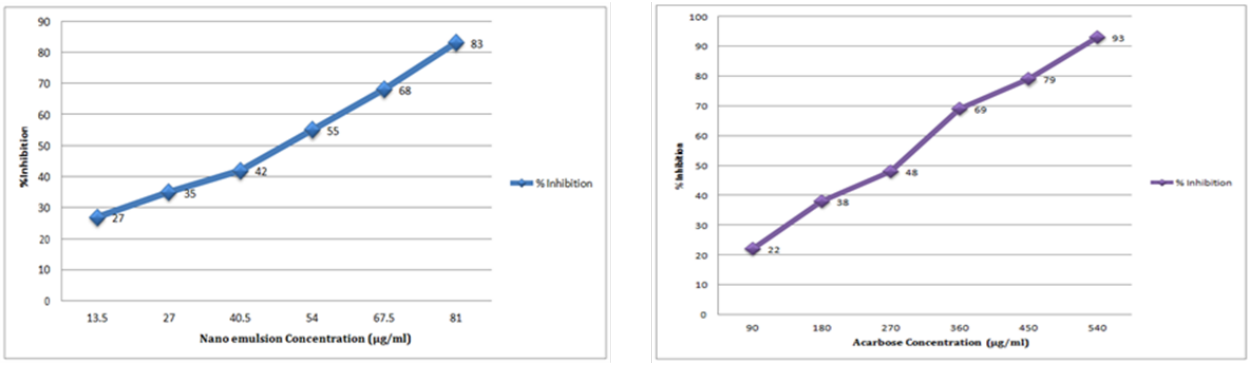

Figure 2: Graph showing α-glucosidase inhibition assay test verses control

Table 1: P Value of inhibition of alpha glucosidase by Mixed fruit juice nanoemulsion and acarbose

	Levene's Teset for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig.(2-tailed)	Mean difference	Std. Error Difference	95% Confidence interval of the difference	
								Lower	Upper
Equal variances assumed	3.020	.157	-242.6	4	.000	-224.6	.92592	-227.2	-222.1
Nano Equal Variances not assumed			-242.6	2.235	.000	-224.6	.92592	-228.2	-221.0

RESULTS AND DISCUSSION

α-glucosidase inhibition assay

Alpha glucosidase inhibitory effect of mixed fruit juice nanoemulsion and acarbose was assessed by the release of p-nitro phenol from PNPG. The IC_{50} values of the formulated fruit juice extracts (49.47 μg/ml) on enzyme inhibition activity were found to be decreased when compared to positive control acarbose (274μg/ml). The maximum inhibition of the formulated nanoemulsion on alpha glucosidase enzyme activity was 83% at 81μg/ml. For the positive control acarbose the maximum inhibition was 93% at 540μg/ml.

Statistical Analysis

Data of the present research were expressed as Mean $\pm$ 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-glucosidse α- amylase, and glucose diffusion inhibition assay are given below.

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 common side effect of acarbose & Metformin that have been reported (Fatima *et al.*, 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 fruit 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, 2011). Hence to overcome this above issue, the raw mixed fruit juice extracts of Nanoemulsion form were used. The present study was designed to investigate the potential effects of traditional medicinal plants- fruits 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 (Gunasekaran *et al.*, 2014).

In our present study, Nano emulsions of mixed fruit juice 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, mixed fruit juice 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 83% at concentration 81μg/ml whereas the standard anti-diabetic drug Acarbose has 93% at concentration 540μg/ml. The study results validate that, the mixed fruit juice 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 IC_{50} 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 27% to 83% at concentration ranging from 13.5μg to 81μg/ml. The obtained results also suggest that the Nano emulsion formulation of mixed fruit juice 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 mixed fruit juice nanoemulsion formulation combines the beneficial effects of all the three fruits such as Phyllanthus emblica, Coccinia grandis and Punica granatum exhibits anti diabetic effect by scavenging free radicals, Inhibition of carbohydrate hydrolyzing enzymes, stimulating insulin secretion and regenerating β-cell architecture.

CONCLUSIONS

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 fruits phyto-constituents and 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 fruits Nano emulsion in our daily 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.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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