AN *IN VITRO* ANALYSIS TO IDENTIFY THE ANTIDIABETIC ACTIVITY OF NATURAL COMPOUNDS BY α-AMYLASE AND α-GLUCOSIDASE ACTIVITY (PIPERINE, LUPEOL AND BETA SITOSTEROL)

Running Title: Invitro analysis to identify the antidiabetic activity of piperine,lupeol and beta sitosterol.
Type of study: Original research

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Abstract
Objective: The objective of the current study is to analyze the in vitro antidiabetic property of natural compounds, Piperine, Lupeol and Beta sitosterol by their inhibitory activity against α- amylase and α-glucosidase enzymes.
Introduction: Piperine is an alkaloid isolated from Piper nigrum, responsible for pungency of black pepper. It is one of the purified natural molecule with bioenhancer properties. Lupeol is a pentacyclic triterpenoid, found in a variety of medicinal plants. It was first isolated from the root barks of Alhagi maurorum. Beta sitosterol is a bioactive phytosterol, naturally present in plant cell membranes. Chemically it's structure is similar to cholesterol. High content of beta sitosterol is present in lipid rich food such as nuts, seed, legumes and olive oil.
Materials and methods: α- amylase and α-glucosidase inhibitory activity of piperine, lupeol and β-sitosterol was examined. Each experiment was performed in triplicates. The results were expressed as percentage inhibition. The data was analysed statistically using one way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the groups. The results with the $p<0.05$ level were considered to be statistically significant.
Results: The results have shown that the lupeol, piperine and beta sitosterol showed a significant increase in the inhibition of α- amylase and α-glucosidase activity in a dose dependent manner with concentrations ranging from 10 to 50µg/ml. It is also observed that beta sitosterol has the maximum anti-diabetic potential followed by piperine and then lupeol.
Conclusion: β-sitosterol has a significant role in the anti diabetic activity compared to Piperine and Lupeol. Further *in vivo* study can be done to develop a potential anti diabetic natural drug.

Keywords: Innovative technique, Antidiabetic activity, Piperine, Lupeol, β sitosterol, α Amylase, α Glycosidase. Diabetes, Novel method,

INTRODUCTION

Diabetes mellitus is a major chronic metabolic disorder characterized by hyperglycemia. The symptoms like polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger) are associated with diabetes mellitus, which ultimately causes various other complications like retinopathy, neuropathy, nephropathy and microangiopathy ((1).
The prevalence of diabetes is increasing globally at a high pace, which was 6.0% of the world's population and expected to be 7.3% in 2025 according to the International Diabetes Federation in 2007 (1). Thus, in order to overcome this increase, there is a need to develop potential medication for diabetes without any side effects. There are a lot of therapeutic mecha- nisms which are responsible for the management of diabetes mellitus, but the one with less or no side effects is preferred mostly.
Drugs from nature play an important role in treatment of different chronic disorders. Natural drugs are more preferred over synthetic drugs due to its natural origin, less side effects, cost effectiveness and effective cure of disease.
Piperine, Lupeol and β-sitosterol are compounds derived from plants. Piperine is an alkaloid isolated from Piper nigrum (black pepper). It has numerous health benefits including antioxidant, antitumor, antihypertensive, anti-asthmatic analgesic, antipyretic, anti-inflammatory, hepato- protective, anti-depressant, anti-bacterial etc (2). Research findings showed the presence of geometrical isomers of Piperine- iso piperine, iso chavicine and chavicine (3). These compounds exhibit numerous pharmacological activities.
Lupeol is another natural bioactive compound. It is a triterpene, widely found in edible fruits, and vegetables. It is present in *Cassia fistula*,(4) in the stem bark of *Crataeva nurvala* and in many other plants also. Previous studies have revealed several important pharmacological effects of lupeol which includes, anti-inflammatory, anti-microbial, anti-protozoal, anti- proliferative, anti-invasive, anti-angiogenic and cholesterol lowering activity. It has

also been tested for its therapeutic efficiency against conditions like, wound healing, cardiovascular disease, kidney disease and arthritis(5).

β sitosterol is the major phytosterol, present in the cell membrane of plants. Its structure is similar to that of cholesterol. It is a potent micronutrient present in higher plants (6). Previous study had found that it possesses antinociceptive, anxiolytic and sedative effects. It also possesses anti-inflammatory, anticancer, hepatoprotective, wound healing effects and protective effects on respiratory diseases (7). Our team has extensive knowledge and research experience that has translate into high quality publications (8),(9),(10),(6),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26)

The current study is done to compare the in vitro antidiabetic property of Piperine, Lupeol and β sitosterol by assessing their inhibitory effect on α- amylase and α -glucosidase enzymes.

MATERIALS AND METHODS

Chemicals and reagents

All chemicals and reagents used in this study were purchased from Sigma Chemical Company St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. Glyphosate was procured from Sigma Chemical Company St. Piperine, Lupeol and beta sitosterol purchased from Sigma Chemical Company St. Louis, MO, USA.

Assessment of in vitro antidiabetic activity

α-amylase inhibitory activity

α-amylase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification (Ademiluyiand Oboh, 2013). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6.8), 10 µl α–amylase (2 U/ml), and 20 µl of varying concentrations of plant extract (10 to µ 50µg/ml) was pre incubated at 37°C for 20 min. Then, the 20 µl of 1% soluble starch (100 mM phosphate buffer pH 6.8) was added as a substrate and incubated further at 37°C for 30 min; 100 µl of the DNS color reagent was then added and boiled for 10 min. The absorbance of the resulting mixture was measured at 540 nm using Multiplate Reader (Robonik). Acarbose at various concentrations (0.1–0.5 mg/ml) was used as a standard. Without test (extract and fractions) substance was set up in parallel as control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula

Inhibitory activity (%) = (1-As/Ac) ×100

Here, As is the absorbance in the presence of a test substance and Ac is the absorbance of control.

α-glucosidase inhibitory activity

α-glucosidase inhibitory activity of extract and fractions was carried out according to the standard method with minor modification (Shai et al., 2011). In a 96-well plate, reaction mixture containing 50 µl phosphate buffer (100 mM, pH = 6. 8), 10 µl alpha-glucosidase (1 U/ml), and 20 µl of varying concentrations of plant extract (0.1 to 0.5mg/ml) was pre- incubated at 37°C for 15 min. Then, 20 µl P-NPG (5 mM) was added as a substrate and incubated further at 37°C for 20 min. The reaction was stopped by adding 50 µl Na2co3 (0.1 M). The absorbance of the released p-nitrophenol was measured at 405 nm using a Multiplate Reader. Acarbose at various concentrations (0.1–0.5 mg/ml) was included as a standard. Without test substance was set up in parallel as a control and each experiment was performed in triplicates. The results were expressed as percentage inhibition, which was calculated using the formula,

Inhibitory activity (%) = (1 − As/Ac) ×100

Where,

As is the absorbance in the presence of the test substance and Ac is the absorbance of control.

Statistical analysis

The data were analysed statistically using one way analysis of variance (ONE-WAY ANOVA). Duncan Multiple range test was used to analyze the statistical significance between groups. The levels of significance were considered at the levels of $p<0.05$.

RESULTS

Alpha amylase inhibitory and alpha glucosidase inhibitory activity of lupeol, piperine and beta sitosterol

Piperine, Lupeol and β-sitosterol exhibit *in vitro* antidiabetic properties. α- amylase and α -glucosidase inhibitory activity of the compounds showed a dose dependent increase in the percentage of inhibition of the enzymes. The percentage of inhibition increases ($p<0.05$) with increase in the concentration of the extract (from 10µg to 50µg).

Among the three compounds β-sitosterol showed significant increase in the antidiabetic activity with the increase in the concentration of extract compared to Piperine and Lupeol (Figure1&2).

Alpha amylase inhibitory activity

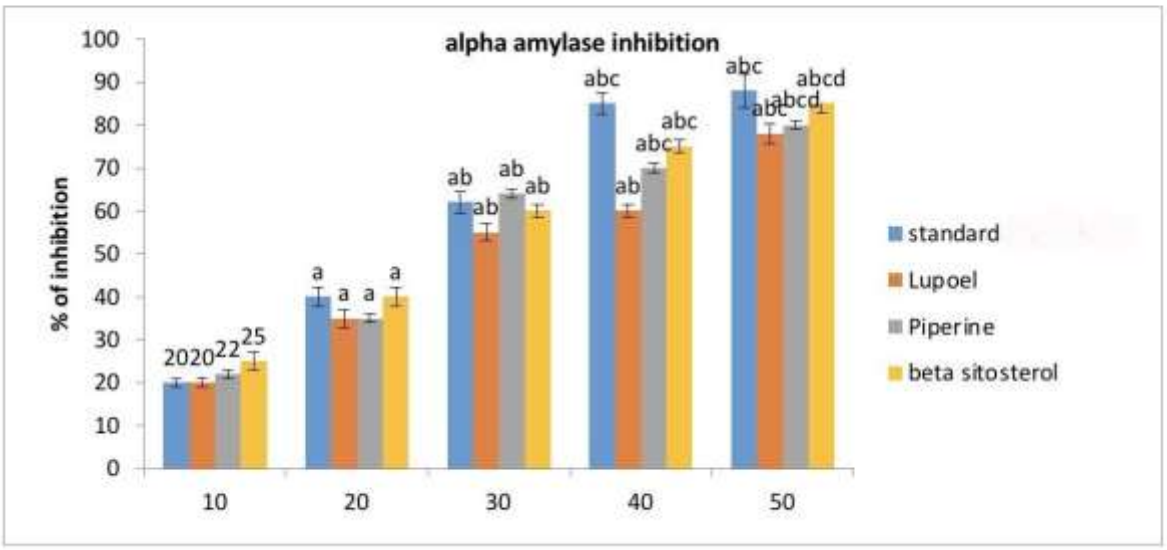

Figure 1: Represents bar graph depicting alpha amylase inhibitory activity of piperine, lupeol, beta sitosterol. X axis represents concentration of the extract in µ g/ml and y axis represents the percentage of inhibition of alpha amylase enzyme. Each bar represents mean ± SD of 6 observations. Significance at the levels of $p < 0.05$. a-compared with 10 µg; b-compared with 20 µg; c-compared with 30 µg.; d-compared with 40µg.

Alpha glucosidase inhibitory activity

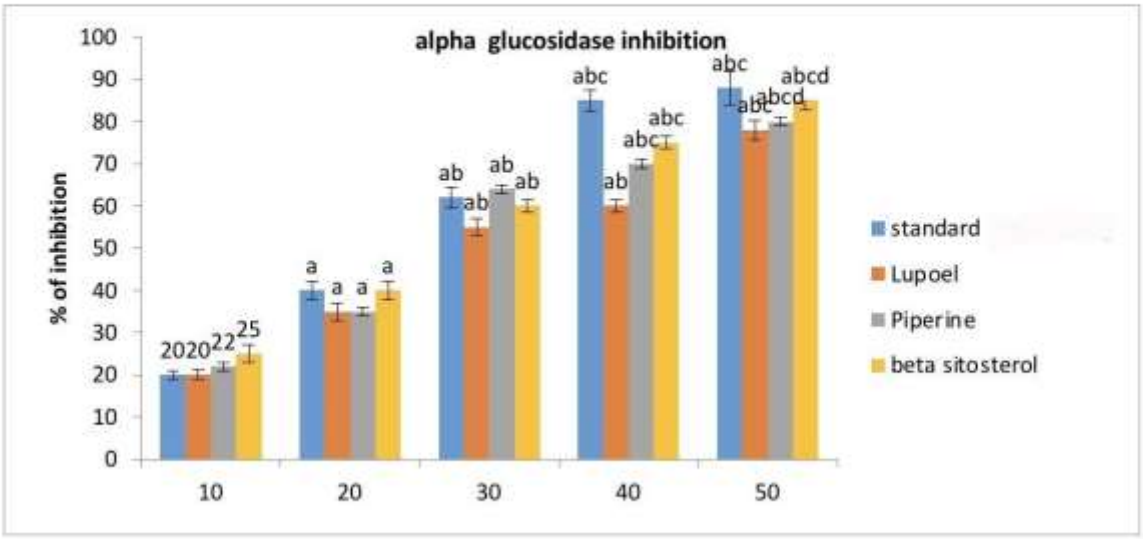

Figure 2: Represents bar graph depicting the alpha glucosidase inhibitory activity of piperine, lupeol and beta sitosterol. X axis represents the concentration of the extracts in µg. Y axis represents the percentage of inhibition of a glucosidase enzyme. Each bar represents mean ± SD of 6 observations. Significance at the levels of $p < 0.05$. a-compared with 10 µg; b-compared with 20 µg; c-compared with 30 µg.; d-compared with 40µg.

DISCUSSION

Diabetes mellitus is one of the world's fastest growing metabolic disorders. Studies have found that appropriately 2.5 – 7% of the world's population is suffering from diabetes and is one of the causes for illness and death of people(2). This disease will lead to severe complications if it is not managed properly. Treatment for diabetes without any side effects is one of the challenges faced by medical professionals. Moreover natural anti diabetic drugs are more accepted besides synthetic drugs by the common people due to its natural origin , fewer side effects and cost effectiveness compared to the available synthetic drugs (27).

The present study focuses on the in vitro antidiabetic property of natural compounds containing Piperine, Lupeol and β-sitosterol. The antidiabetic property was evaluated by the percentage of inhibition of α Amylase and α Glucosidase enzymes by these natural compounds.

α Amylase is an enzyme present in the human body which converts complex polysaccharide into oligosaccharides and disaccharide (28). The disaccharides are further hydrolyzed into monosaccharide by α glucosidase enzyme (29) and is absorbed through the small intestine into the hepatic portal vein thereby increasing the postprandial glucose level (30). The inhibitory actions of these enzymes decreases the blood glucose level.

Piperine, Lupeol and β sitosterol were found to inhibit the action of α Amylase and α Glucosidase (31,32). This shows that these compounds have potential anti-diabetic properties. A similar study conducted by Gurupriya et al found that Lupeol extracted from methanolic extract of Andrographis echioides exhibits significant α Amylase and α Glucosidase inhibitory activity (33). They suggested that Lupeol is potential source of natural anti-diabetic compound. Another study reported that lupeol present in the ethanolic extract of flowers of banana have in vitro and in vivo antihyperglycemic activity (34). Similarly Gupta et al studied the antidiabetic and antioxidant potential of Lupeol in experimental hyperglycaemia (35). The above findings agree with the present study stating that lupeol possesses potential anti-diabetic property.

Piperine, an alkaloid derived from the plant (Piper nigrum) has multiple health benefits (36), one among them is the anti-diabetic property. The current study evaluated alpha amylase and alpha glucosidase inhibitory activity of Piperine and found that there is a dose dependent increase in the percentage of inhibitory activity. Percentage of inhibition increases with increase in the concentration of the extract (from 10 µg/ml to 50 µg/ml). This shows the anti diabetic activity of piperine. Haq et al has reviewed the biological effects of piperine and the function of piperine in promoting glucose uptake in skeletal muscles (2). Similar study conducted (37) on the impact of curcuminoids plus piperine administration in type 2 diabetic patients also found the same results as mentioned above. Thus previous literature agrees with the present study and thereby adds to the consensus.

The current research also focused on the antidiabetic property of β-sitosterol. It also showed an increase in the percentage of enzyme inhibition with the increase in concentration. Previous study (6) conducted on the pharmacological effects of beta sitosterol showed that it is a potential nutraceutical for the treatment of diabetes. Rethinam et al conducted a study to find the bioactive compound present in Coldenia procumbens, a plant with antidiabetic property, and found the compound as beta sitosterol ((6,38). The above mentioned previous studies are in accordance with the present study in which there showed a potent inhibition towards the activities of alpha amylase and alpha glucosidase enzymes, which showed the in vitro antidiabetic potential of the compound.

In the present study, the comparative analysis of antidiabetic potential among the three compounds showed that beta sitosterol showed higher antidiabetic properties and it decreases in the order β-sitosterol> piperine>lupeol.

The limitations of the current study was that, since the raw material used for the study were natural products, they might not be found everywhere and also they may get damaged during its culture in laboratory

Future scope

Piperine, lupeol and beta sitosterol, can be used as therapeutic drugs for the treatment of diabetes after further invivo studies.

CONCLUSION

It is concluded from the present finding that piperine, lupeol and beta sitosterol have anti-diabetic properties. Among the three, beta sitosterol has a potential role and hence it can be used as a therapeutic drug for diabetic treatments.

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AUTHOR CONTRIBUTION

A) Reenu Joshy- contributed in designing the study, execution of the project, statistical analysis, manuscript drafting.
B) V. Vishnu Priya- contributed in study design, guiding the research work, manuscript correction.
C) J. Selvaraj- contributed in study design, statistical analysis, manuscript proofreading and correction.
D) Gayathri. R- contributed in study design, statistical analysis, manuscript proofreading and correction.
E) Kavitha S- contributed in study design, statistical analysis, manuscript proofreading and correction.

CONFLICT OF INTEREST

The authors hereby declare that there is no conflict of interest in this study.

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