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Full Length Original Research Paper

Antidiabetic effect of Ethanol extract of *Syzygium jambolanum* seed (In-Vitro)

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Abstract: Plant *Syzygium Jambolanum* is a well known Indian folklore medicine for treatment of diabetes mellitus. They have also reported to possesses anti-bacterial and anti-inflammatory activities .The in-vitro study was undertaken to investigate and confirm antidiabetic activity of the ethanol extract of the seeds using yeast model and to determine a-amylase inhibition. Percentage increase in glucose uptake by yeast was seen by varying; extract concentration and time. At high extract concentration yeast shows decrease in glucose uptake but shown increase in uptake at lower extract concentrations. It also showed very high percentage inhibition of a- amylase activity in a dose dependant manner when compared with a standard drug, Acarbose.

Keywords: Syzygium jambolanum, yeast, a- amylase assay, ethanol extract, Acarbose

Introduction:

Diabetes mellitus is a group of metabolic diseases in which a person has high blood sugar either because the pancreas does not produce enough insulin for the balance of glucose level in the blood stream or because cells do not respond to the insulin that is produced. This is characterized by chronic hyperglycaemia with disturbance of carbohydrate, fat and protein metabolism [1]. People with diabetes mellitus are considered to be at high risks of atherosclerotic, cardiovascular disease [2, 3] renal failure, blindness and limb amputation [4]. Antidiabetic therapy is to reduce hyperglycaemia which is the result of decreased insulin sensitivity or decreased insulin secretion from pancreatic β-cells which can further inhibit insulin secretion from pancreas and diminish insulin mediated glucose uptake in peripheral tissue [5]. Various medicinal plants continue to play an important role in the treatment of diabetes for example *Eugenia jambulana, Ficusracemosa, Tinosporacardifolia*, *Gymnemasylvestrae* and *Aeglemarmelos*, more than 1123 plant species have been used ethno pharmacologically or experimentally to treat symptoms of diabetes [6]. It is commonly known as black plum and originally indigenous to India, is one of the important antidiabetic plant [7].Mainly all part of the plant like leaf, bark etc. already used as an antidiabetic treatment in the form of ayurvedic medicine, particularly in developing countries where most people have limited resources and do not have an access to modern treatment [8]. The seeds of these plants also show antibacterial and antifungal activity [9]. This is native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, the Philippines, and Indonesia. With this background, the present study was planned to evaluate the effect of *Syzygium jambolanum* plant seed ethanol extract,

carbohydrate hydrolysing enzymes and glucose uptake by yeast cells.

Materials and methods:

1. Plant material:

The fruit of *Syzygium Jambolanum* were collected from Vellore, Tamil Nadu in India on 8th of August 2012. The plum was removed and seeds were dried in hot air oven at 45°C.

2. Method of extraction

The seeds were crushed into small pieces, air-dried and powdered. Powdered sample (100gm) were mixed with 70% ethanol (500ml) and kept in orbital shaker for 24 hour. After 24 hours it was filtered with Whatman Grade No. 1 Filter Paper followed by concentrating and drying in rotary evaporator at 40°C. Soxhlet extraction process was avoided to prevent denaturation of heat labile compounds as the boiling point of ethanol is 78.37 °C. The yield of ethanol extract was 28.7gm per 100gm of seed powder. The extract was kept at deep freeze temperature for further use.

3. Glucose uptake by yeast

Commercial baker's yeast was washed by repeated centrifugation (3,000×g; 5 min) in distilled water until the supernatant fluids were clear and a 10% (v/v) suspension was prepared in distilled water. Various concentrations of extracts (1–5 mg) were added to 1 ml of glucose solution (1gm/ml) and incubated together for 10 min at 37 °C. Reaction was started by adding 100µl of yeast suspension, vortex and further incubated at 37 °C for 60 min. After 60 min, the tubes were centrifuged (2,500 × g, 5 min) and glucose was estimated in the supernatant by DNSA method. The incubation time was also varied ranging from 1 hour to 5 hours to see the effect of incubation time.

The same experiment was carried out using serially diluted 1gm/ml extract from 10^{-1} to 10^{-10}. Glucose uptake was calculated by above formula. All the experiments were carried out in triplicates. [10]

4. a- Amylase assay

The a -amylase inhibitory activity was determined by an assay modified from the Worthington Enzyme Manual (Worthington Biochemical Corp.1993a), with some modifications. [11]

Statistical Analysis- All determinations in this article were carried out in triplicates and data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences using SPSS 14.0 software. Values were considered significant at $p \leq 0.05$.Graphs were plotted using Microsoft power point.

Results and Discussion

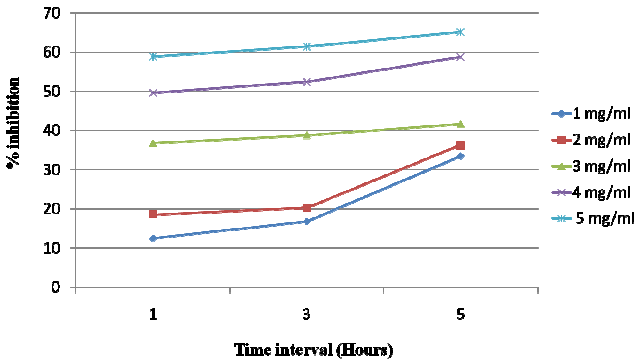
1. Glucose uptake by yeast

Fig. 1shows the increase in percentage inhibition of glucose uptake by yeast cells with extract concentration ranging from 1mg/ml to 5mg/ml. The amount of glucose remaining in the medium after a specific time serves as an indicator of the glucose uptake by the yeast cells. At lower concentration i.e. 1mg/ml the increase in percentage inhibition was somewhat linear but as concentration increases higher till 5mg/ml it tends to become somewhat more exponential.

Glucose uptake in yeast cells (*Saccharomyces cerevisiae*) is rapid and occurs down the concentration gradient. Glucose uptake reaches equilibrium and is not accumulative.

Phosphorylation accompanies with glucose entry into the cell. Glucose transporters are stereospecific for certain hexoses and carries glucose, fructose and mannose [12, 13]. This behaviour of decrease in uptake by yeast cell may be attributed to anti microbial activity of extract at higher concentrations which either killed yeast cell or inhibited its growth and metabolism. [14]

Fig. 1: Percentage inhibition of glucose uptake



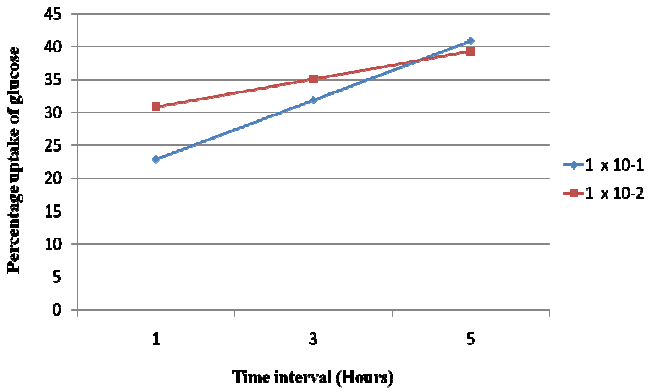
(P<0.05 when compared to control, Values are expressed as mean ± SEM. Then plotted in graph)

From Fig. 2 and Fig. 3 represents percentage increase in glucose uptake. In Fig. 2 and Fig. 3 it can be seen that lower concentration of extract shows increase in glucose uptake by yeast cells. Increase in glucose uptake occurred in an interesting way. In Fig. 2 for dilution 10^{-1} to 10^{-2}, percentage of glucose uptake slowly happens and increases with time. But for dilution glucose $10^{-3}$and 10^{-10} percentage of glucose uptake is high in the beginning and then decreases with time.

From Fig. 2 it can be inferred that dilution 10^{-1} to 10^{-2}increases glucose uptake in yeast cells consistently and sustainably whereas in Fig. 3 for higher dilution it affect decreases along with time.

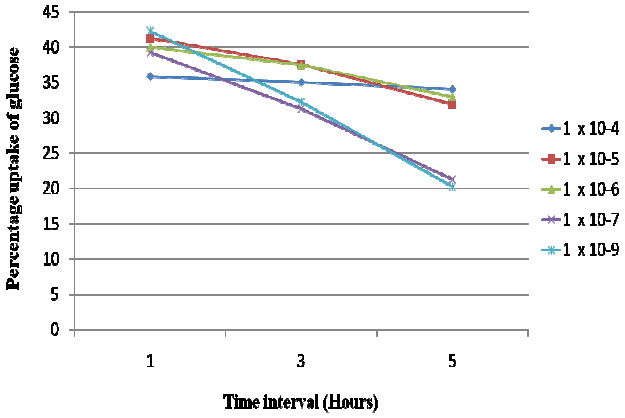
Some of the data was plotted in the graph to increase clarity and avoid crowing.

Fig. 2: Percentage increase in glucose uptake



(P<0.05 when compared to control, Values are expressed as mean ± SEM. Then plotted in graph)

Fig. 3: Percentage increase in glucose uptake



(P<0.05 when compared to control, Values are expressed as mean ± SEM. Then plotted in graph)

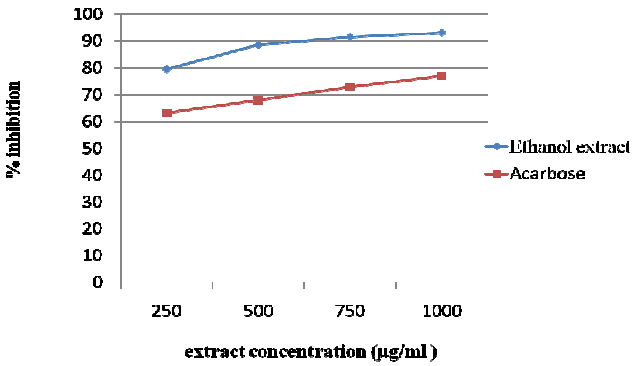
2. a- amylase assay:

Fig. 4 shows percentage inhibition of a-amylase by *Syzygium jambolanum* seed ethanol extract and Acarbose. Both potentially inhibit a-amylase activity on starch in vitro. As the concentration of each increase percentage inhibition also increases. Extract shows even more percentage of inhibition of a-amylase in-vitro, ranging from 79.41% to 93.06% for 250 to 1000µg/ml concentration compared to 63.24% to 77.07% for same concentration of Acarbose.

Acarbose, a standard antidiabetic drug, competitively and reversibly inhibits pancreatic a-

amylase and intestinal brush border a-glucosidases, resulting in retardation of glucose absorption from hydrolysed complex carbohydrates and reduction of blood-glucose concentration [15]. Extract can be expected to show same hypoglycaemic effect in-vivo at even lower concentration as shown by the standard drug.

Fig. 4: a- amylase inhibition



Conclusion:

It may be concluded that increase in concentration of extract of *Syzygium Jambolanum* seed inhibits the glucose uptake and as time of incubation increases, inhibition increases even more. So effect of extract on glucose uptake by yeast is dependent of concentration and time of incubation. Extract is potent in a-amylase inhibition in vitro at even lower concentration and also increases with increase in concentration. So, crude ethanol extract of *Syzygium Jambolanum* seed shows hypoglycaemic effect by increasing glucose uptake by yeast cells and inhibiting a-amylase activity on starch in vitro at lower concentration.

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