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## COMBINED IN-VITRO ANTIDIABETIC POTENTIAL OF *SALACIA OBLONGA* AND *ENICOSTEMMA LITTORALE*

DUGGAL S\* AND MITTAL SK

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### ABSTRACT

**Introduction:** Indian Medicinal plants have been used from long centuries back for the treatment of diabetes mellitus. The literature review reported the antidiabetic activity of *S. oblonga* Linn. stem and *E. littorale* Blume. aerial part against various models of diabetes. **Materials and Methods:** The present study evaluated the combined *in-vitro* antidiabetic potential of aqueous extract on glucose adsorption capacity, glucose diffusion using dialysis bags and glucose uptake by yeast cells. **Results:** The extracts adsorption capacity was related to the molar concentration of glucose. The glucose bound ratio was 73.21, 81.11, 94.09 and 97.45 with aqueous extract of *S. oblonga*, *E. littorale*, combined *S. oblonga* & *E. littorale* and standard drug (Acarbose), respectively from a 100 mM glucose concentration solution. The results of *in-vitro* glucose diffusion studies showed that the aqueous extracts decrease the transport of glucose in dose dependent manner. The combined extract of plants had showed comparable glucose adsorption to acarbose. A significant difference ( $p < 0.05$ ) in the diffusion of glucose were observed when result compared with individual extract of plants. Further, dose dependent effects were found in glucose uptake by yeast cells assay, but there was an inverse relationship between the concentration of glucose and dose of plant extracts. **Conclusion:** The present study indicates that the aqueous extract of *S. oblonga* and *E. littorale* is able to adsorb glucose molecules and inhibit glucose transport, which makes them less available for absorption in the intestine. Further, research has to be carried out in order to find out active biochemicals to facilitate their use to control hyperglycemia.

**Keywords:** Medicinal plants, Glucose transport, Glucose adsorption, Yeast cell, Antidiabetic

## INTRODUCTION

Diabetes mellitus is a multifactorial metabolic condition that is becoming more prevalent around the world. It is characterised by an increase in blood glucose level caused by either a deficiency in insulin secretion, irregularities in insulin action, or both [1, 2]. Diabetic complications have become a leading source of morbidity and mortality [3, 4]. According to Cho *et al.*, there were 135 million diabetics in 1995 and by 2045, that number is expected to jump nearly 693 million [5]. Furthermore, due to obesity, population growth, poor diets, and sedentary lifestyles, a major percentage of this increase will occur in developing countries. It has more than doubled in prevalence over the last two decades, making it the country's fifth-deadliest disease, causing roughly four million fatalities in 2017 globally [6, 7]. Modern synthetic anti-diabetic medications have real side effects, such as renal, liver, and haematological complications [8]. Diabetes treatment costs a lot to people and their families, as well as national and international health systems [9, 10]. In India, the majority of the population (80%) relies on the Indian Alternative System of Medicine since it is widely available, inexpensive, and has few or no side effects [11].

There are numerous medicinal plants in both traditional and modern medicine that have contributed to the treatment of diabetes mellitus. Literature review showed plants such as *S. oblonga* and *E. littorale* have an important function in human health. The *S. oblonga* and *E. littorale* are also known as "Saptrangi" and "Chota-chiretta," respectively, and are found all over India. Both the plants are used to treat diabetes along with various ailments such as hernia, hyperlipidemia, rheumatism, abdominal ulcers, insect poisoning, itching, swelling, asthma, inflammation, gonochea and cancer in folk medicines [12-14]. *Salacia* spp. has been utilised in the form of food supplements and formulations in nations such as India, China, Japan, Korea, and several eastern countries to treat diabetes mellitus [15]. Kotalanol, a powerful natural  $\alpha$ -glucosidase inhibitor was derived from *Salacia*, has higher inhibitory action against disaccharide than salacinol and acarbose [16].

According to the Indian Council of Medical Research's nutritional analysis report, 100 grammes of fresh plant of *E.littorale* contain 4.2 grammes of fiber [17]. However, previous literature review had showed inhibitory potential of *S. oblonga* and *E. littorale* extracts against  $\alpha$ -amylase and  $\alpha$ -

*glucosidase* enzyme, which makes glucose less available for absorption and therefore, interfere in the glucose diffusion in the small intestine [18-21]. Medicinal plants are used singly or in combination. The Polyherbal are plant-based formulations, which might apply synergistic, potentiative, agonistic activities by uprightness of its related different dynamic principles [22]. From this point of view, efforts have been made to examine the combined effect of aqueous extracts of *S. oblonga* Linn. stem and *E. littorale* Blume. aerial part on *in-vitro* glucose adsorption capacity, glucose diffusion and glucose uptake by yeast cell. This combined study provides valuable information for future research to formulate effective and safer alternative medicines in the treatment of diabetes mellitus.

## MATERIALS AND METHODS

### Collection and identification of plants

The plants were collected from the local market in a dried form and authenticated by Dr. A. Singh, Dhanvantri Ayurvedic College, Mohali. Other chemical used during the experiments was procured from HIMEDIA laboratory, Mumbai, India. Distilled water was used for the preparation of the different solutions.

### Preparation of the extracts

The dried plant was grinding by mechanical grinder and pass through a 40-mesh sieve. The 100 g powder material was extracted with water for 30 minutes at 50°C. The extract was centrifuged, filtered, and crude powder extract were dried on the water bath, which was further used for the evaluation of *in-vitro* antidiabetic activities.

### Determination of glucose adsorption capacity

The herbal extracts glucose adsorption capacity was evaluated by the method of Ou *et al.*, with slight modification [23]. The reaction mixture was prepared by addition of plant extracts (200 mg) and 40 ml of glucose solution of different concentrations (5, 10, 20, 40, 60, 80 and 100 mmol/l). The reaction mixture was mixed and incubated in a shaker water bath for 6 hrs at 37°C. The reaction mixture was centrifuged for 15 minutes at 4800 rpm. The supernatant was separated for the estimation of glucose concentration. The standard reaction mixture was prepared with addition of acarbose (200 mg) in place of plant extract. The bound glucose concentration was calculated by following formula:

$$\text{Glucose Bound} = \frac{G1 - G6}{\text{Weight of Sample}} \times \text{Volume of Solution}$$

Where, G1 is the glucose concentration of original solution. G6 is the glucose concentration after 6hr.

#### **Determination of *in-vitro* glucose diffusion**

The *in-vitro* glucose diffusion was determined using methods employed by Ahmed [24]. The reaction mixture was prepared by mixing a 25 mmol/l glucose solution (25 ml) with a 1 ml solution of plants extract (100 mg/ml & 200 mg/ml). The reaction mixture was poured to the dialysis bages. The dialysis bags transfer to

flask containing 250 ml of distilled water, further kept at 37°C in a water bath with shaker. The concentration of glucose in the dialysate was determined using the glucose oxidase peroxidase technique at 30, 60, 90, 120, 150, and 180 minutes. The reaction for standard and blank was prepared using acarbose (200 mg) and distilled water respectively. The glucose dialysis retardation index was calculated using the formula below:

$$\text{GDRI (\%)} = 100 - \frac{\text{Glucose content with addition of sample (mg/dl)}}{\text{Glucose content of control (mg/dl)}} \times 100$$

#### **Determination of glucose uptake by yeast cells**

The method of Cirillo, with slight modification was used for the preparation of yeast cell reaction mixture [25]. Washed the commercial baker's yeast in distilled water with repetitive centrifugation for 5 min at 4200 g till the supernatant fluid was clean. Discard the supernatant fluid and a 10% (v/v) suspension was made with yeast cell pellet using distilled water. 1 ml of aqueous extracts of concentration (100-500 µg/ml) were added to 1 ml of glucose solution (5, 10, and 20 mmol/l) separately and incubated

for 10 minutes at 37°C. The reaction was initiated by addition of 100 µl of yeast suspension in each extract-glucose reaction mixture. The reaction mixture was vortexed and incubated for 60 minutes at 37°C. Following 60 minutes, centrifuged the tubes for 5 minutes at 3800 g. The supernatant was collected for the estimation of glucose. The standard reaction solution was prepared by addition of acarbose (100-500 µg/ml) in place of plant extract. The percent increase in glucose uptake was determined using the following formula:

$$\text{Increase in glucose uptake (\%)} = \frac{\text{Absorbance control} - \text{Absorbance sample}}{\text{Absorbance of control}} \times 100$$

## STATISTICAL ANALYSIS

All values were indicated as mean $\pm$ SD and the data were collected in triplicate. The data were analysed by analysis of variance (ANOVA) with turkey multiple comparison test using Graph Pad Prism, ver 5.00 software. At 95% confidence interval,  $p < 0.05$  was considered as statistically significant.

## RESULTS AND DISCUSSION

### Effect of aqueous extract on glucose adsorption capacity

**Table 1** shows the findings of aqueous extracts of *S. oblonga* and *E. littorale*. The aqueous extracts of both the plants had showed a significant effect on glucose adsorption. Furthermore, the glucose adsorption capacity of *E. littorale* aqueous extract was found to be higher than that of *S. oblonga* aqueous extract. The present investigation found that the combined impact of both extracts in a 1:1 ratio produces a greater affect on glucose adsorption than individual extracts. The extract's combined impact was comparable to that of standard acarbose. *S. oblonga*, *E. littorale*, combined extract (1:1 ratio), and standard medication Acarbose had adsorption capacities of 73.21, 81.11, 94.09, and 97.45 at 100 mM glucose concentration, respectively. The glucose adsorption capability of both the aqueous extracts was shown to be dependent on

glucose content. According to Das and Devi, the postprandial blood glucose level may be reduced by the adsorption of glucose in the intestinal lumen (26). The glucose adsorption capacity of aqueous extracts extract might be due to the presence of fibers. The present result supported by the research conducted by ICMR, which states *E. littorale* 100 gm of fresh plant contains 4.2 gm of fibers [17].

### Effect of aqueous extract on glucose diffusion and glucose dialysis retardation index (%)

The effect of aqueous extracts on glucose diffusion and percentage glucose dialysis retardation index was shown in **Table 2 and Table 3** respectively. Although the rate of diffusion of glucose in presence of individual extracts, combined aqueous extract and standard drug Acarbose had showed significantly difference ( $p \leq 0.05$ ,  $p \leq 0.01$ ,  $p \leq 0.001$ ) than the control group at different time intervals (30-180 minutes).

The result of present study indicated dose dependent inhibition in the rate of diffusion of glucose.

In general, the rate of diffusion was decreased in presence of acarbose containing group at 200 mg/ml than the individual and combined aqueous extract groups of *S. oblonga* and *E. littorale*. The combined extract (1:1 ratio) of plants had showed

comparable inhibition in glucose diffusion to standard drug acarbose. At 180 min, the rate of inhibition of glucose diffusion was  $0.99 \pm 0.018$  and  $0.83 \pm 0.011$  with combined extract and standard drug acarbose respectively.

A dose dependent inhibition in percentage of glucose dialysis retardation index was observed in the present study. Although the results indicate that the combined extract in (100 mg + 100 mg) had showed comparable glucose dialysis retardation index with standard drug acarbose. The percentage of GDRI was high in first 30 min as compared to other time intervals in all the treatment groups. The results was showed that the effect of aqueous extracts of *E. littorale* at different dose levels (100 mg & 200 mg) were more consistent as compared to *S. oblonga* extract up to 120 minutes.

The presence of fibers can help to reduce postprandial hyperglycemia through different mechanism. They may increase the viscosity of small intestinal fluids, making glucose transport into the bloodstream more difficult. Furthermore, glucose may attach to these fibers, lowering their concentration in the small intestine lumen. Finally, fibers may inhibit  $\alpha$ -amylase and  $\alpha$ -glucosidase, which further reduces the postprandial increase in

blood glucose level by inhibiting the digestion of starch [16, 21, 23, 27].

#### **Effects of aqueous extract on glucose uptake by yeast cells**

The rate of transport of glucose across the cell membrane in yeast cells was given in **Table 4**. The treatment with aqueous extract of *S. oblonga* stem and *E. littorale* aerial part had showed a dose dependent effect on glucose uptake by yeast cell. Glucose absorption by yeast cells increased in inverse proportion to concentration of glucose in all the test groups and standard drug acarbose. The yeast cell glucose uptake percentage was decreased with increased glucose concentrations to 5, 10, and 20 mM. *S. oblonga* aqueous extract in 5 mM glucose medium, had showed 63.31%, 64.65%, 72.22%, 83.71%, and 90.16%, glucose uptake at 100-500  $\mu$ g/ml concentration respectively. Whereas, same extract in 20 mM glucose medium had showed 10.77%, 16.33%, 20.36%, 23.63%, and 26.41%, glucose uptake at different concentration. The percentage of glucose uptake was high in aqueous extract of *E. littorale*, when compared with *S. oblonga* extract. There was 59.62%, 68.42%, 74.84%, 85.92%, and 96.53%, glucose uptake at 100-500  $\mu$ g/ml concentration respectively with aqueous extract of *E. littorale* in 5 mM glucose

medium. The percentage increase in glucose uptake was 21.39%, 24.21%, 27.18%, 33.11% and 39.26%, at 100-500  $\mu\text{g/ml}$  concentration respectively in 20 mM glucose medium.

The *in-vitro* antidiabetic activities of aqueous extract of *S. oblonga* stem and *E. littorale* aerial part may be due to the presence of bioactive constituents [28-31]. Previous

studies have shown that the active transport of glucose by glucose transporters in the small intestine can be influenced by many hypoglycemic factors such as polyphenol [32], insulin [33] and gastrointestinal motility [34]. The result of the present study indicated the direct proportional relationship with the molar concentration of glucose.

Table 1: Glucose Adsorption Capacity of Aqueous Extract

Glucose Concentration	<i>S. oblonga</i>	<i>E. littorale</i>	Combined <i>S. oblonga</i> + <i>E. littorale</i>	Acarbose
Glucose Adsorption Capacity mM				
5 mM	2.11±0.14	3.21±0.36	4.02±0.81	4.74±0.37
10 mM	4.21±0.37	5.66±1.60	6.25±1.12	8.92±1.44
20 mM	12.21±0.68	15.21±1.37	17.82±2.42	18.27±1.31
40 mM	26.21±1.81	29.72±2.61	35.61±2.72	37.58±2.47
60 mM	37.21±1.57	41.53±1.49	52.42±1.58	58.77±1.88
80 mM	64.21±2.96	69.84±2.73	72.28±2.09	79.22±1.29
100 mM	73.21±1.88	81.11±2.83	94.09±2.21	97.45±2.67

Note: Values are expressed as Mean±SD, (n= 3)

Table 2: Glucose Diffusion Effect of Aqueous Extract

Group (Dose)/ Time	30 min	60 min	120 min	180 min
Glucose Content in Diasylate (mM)				
Control group	0.99±0.019	1.41±0.022	1.99±0.013	2.04±0.024
<i>S. oblonga</i> (100mg/ml)	0.77±0.018	1.24±0.016	1.76±0.011*	1.84±0.022*
<i>S. oblonga</i> (200mg/ml)	0.54±0.016	0.83±0.019*	1.24±0.015#	1.43±0.017#
<i>E. littorale</i> (100mg/ml)	0.51±0.011*	0.76±0.012*	1.16±0.020#	1.25±0.015#
<i>E. littorale</i> (200mg/ml)	0.42±0.021#	0.63±0.015#	0.92±0.014#	1.17±0.018#
Combined <i>S. oblonga</i> & <i>E. littorale</i> (100 & 100mg/ml)	0.33±0.067#	0.51±0.015 <sup>o</sup>	0.77±0.014 <sup>o</sup>	0.99±0.018 <sup>o</sup>
Acarbose (200mg/ml)	0.29±0.014#	0.59±0.012 <sup>o</sup>	0.71±0.013 <sup>o</sup>	0.83±0.011 <sup>o</sup>

Note: Values are expressed as Mean±SD, (n= 3) one way ANOVA followed by Turkey's multiple comparisons (\*p≤0.05, #p≤0.01, p≤0.001<sup>o</sup>)

Table 3: Glucose Dialysis Retardation Index Percentage (GDRI) of Aqueous Extract in Glucose Diffusion

Group (Dose)/ Time	30 min	60 min	120 min	180 min
Glucose Dialysis Retardation Index (%)				
<i>S. oblonga</i> (100mg/ml)	22.22±0.018	14.28±0.016	10.52±0.011	10.00±0.022
<i>S. oblonga</i> (200mg/ml)	45.45±0.016	42.85±0.019	36.84±0.015	30.00±0.017
<i>E. littorale</i> (100mg/ml)	48.48±0.011	42.85±0.012	42.10±0.020	40.00±0.015
<i>E. littorale</i> (200mg/ml)	57.57±0.021	57.14±0.015	52.63±0.014	45.00±0.018
Combined <i>S. oblonga</i> & <i>E. littorale</i> (100 & 100mg/ml)	66.66±0.052	64.28±0.011	63.15±0.018	55.00±0.021
Acarbose (0.2g/ml)	70.70±0.014	64.28±0.012	62.15±0.013	55.00±0.011

Note: Values are expressed as Mean±SD, (n= 3)

Table 4: Effect of Aqueous Extract on Glucose Uptake by Yeast Cells

Sample (Glucose)/ Dose	100µg/ml	200µg/ml	300µg/ml	400µg/ml	500µg/ml
Increase in Glucose Uptake (%)					
<i>S. oblonga</i> (5 mM)	63.31±1.02	64.65±2.33	72.22±2.31	83.71±3.63	90.16±3.21
<i>S. oblonga</i> (10 mM)	33.26±2.32	41.92±1.40	49.19±2.09	56.35±3.22	61.94±2.86
<i>S. oblonga</i> (20 mM)	10.77±2.18	16.33±1.65	20.36±1.37	23.62±2.86	26.41±1.23
<i>E. littorale</i> (5 mM)	59.62±1.76	68.42±2.88	74.82±2.53	85.91±3.06	96.03±1.99
<i>E. littorale</i> (10 mM)	38.41±1.81	49.73±2.07	57.61±2.77	64.45±1.72	77.61±2.61
<i>E. littorale</i> (20 mM)	21.39±1.54	24.21±2.11	27.18±1.29	33.11±2.66	39.26±2.38
Acarbose (5mM)	82.11±2.81	87.66±3.04	93.32±3.44	95.08±2.05	96.17±1.05

Note: The data given as Mean±S.D, (N=3)

## CONCLUSION

An extract of the plant has indicates good glucose adsorption and glucose diffusion retardation potential, demonstrated its potential to decrease the availability of glucose for diffusion into the blood stream. On the basis of these results, we concluded that the aqueous extract of *S. oblonga* and *E. littorale* individually and in combination was able to reduce the postprandial hyperglycemia through enhancement of glucose adsorption capacity, by reducing the rate of glucose diffusion in small intestine and increasing the glucose utilization by yeast cell. The results from the different researcher also supported that herbal drug may produce their hypoglycemic activities by combination of different mechanism. Further *in-vitro* and *in-vivo* activities in research are necessary to find out exact mechanism of action and long term safety of herbals, when prescribe in combination.

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