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Inhibition of α-glucosidase enzyme by *Parsley crispum* L. leaf extract: Possible role in controlling postprandial hyperglycemia

Omar Al- harbi[1], Shalam Mohamed Hussain[2]*

[1]*King Abdulaziz Medical city , Riyadh, KSA*

[2]*Department of Pharmacology, College of Pharmacy, Qassim University, KSA*

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Abstract:

Inhibition of intestinal α glucosidase for preventing rise in postprandial glucose level in diabetics forms an integral part of the management of diabetes. Parsley leaves extract (PLE) in the present study was explored for the same. The chemical analysis of extract revealed the presence of tannins, flavonoids, sterols and triterpenes. In this preliminary study PLE showed promising effects in in vitro test using isolated α glucosidase enzyme from rat intestine and in vivo study using different doses of PLE were carried against a sucrose load of 2.5 g/kg body weight in rats. The maximum inhibition of α-glucosidase produced by the extract was upto 80%. The extract exhibited a dose dependent reduction in glucose level with a maximum response being observed was shown by 400 and 800 doses of PLE at intervals of 120 and 180 mins. This effect of PLE indicates that it has a potential in preventing postprandial hyperglycemia. In conclusion the extract of parsley leaves possesses the excellent antihyperglycemic effect and this may be mediated by α glucosidase inhibition. These results suggest that PL extract can provide an effective remedy for managing postprandial hyperglycemia in a more safe and cost effective manner.

Keywords: Parsley leaf extract, Postprandial hyperglycemia, Inhibitor of α glucosidase enzyme, Diabetes mellitus

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder caused by an absolute or relative lack of/or resistance to insulin and is characterized by hyperglycemia in the postprandial and/or fasting state. Presently there is an estimated 150 million people worldwide with diabetes and that the number is likely to increase to 300 million by 2025. Postprandial hyperglycemia in diabetes mellitus could induce non enzymatic glycosylation of various proteins, resulting in the development of chronic complications. Therefore controlling postprandial glucose is critical in the early treatment of diabetes mellitus as well as in reducing chronic vascular complications which can be achieved through inhibition of intestinal α-glucosidases causing delay in carbohydrate absorption. α -glucosidase is a membrane bound enzyme at the epithelium of the small intestine that hydrolyses the cleavage of glucose from disaccharides and oligosaccharides [1-3].

Screening of α glucosidase inhibitors from plants and synthetic sources is increasing because of their usefulness in managing diabetes mellitus. The leaves and seeds of the herb, Parsley L are culinary as well as medicinal herb. They are a functional food for their unique antioxidants and disease preventing properties. This biennial herb is native to the Mediterranean region; and belongs to the family of apiaceae of the genus; Petroselinum, and is known as Petroselinum crispum. This herb is a small plant with dark green leaves resembling coriander, however, has a milder flavor than coriander. In traditional medicine, it is used for treatment of digestive disorders, and dyslipidemia, flatulence, insomnia, renal disorders, loss of appetite and as a diuretic. Studies have demonstrated diuretic, ulcer protective effects and hypolipidemic, anti-inflammatory. A large number of compounds have been isolated from parsley, including flavonoids, euginol, polyphenols, cineole, citronelol, coumarins and hydroxy coumarins [4,5].

*Corresponding author *E - mail:* DrShalamMohamed@qu.edu.sa

Parsley has been reported for its antidiabetic effect but through this study we intend to explore its mechanism via inhibition of α-glucosidase enzyme in preventing post prandial hyperglycemia [5,6]. The synthetic antiα-glucosidase drug, acarbose, was used as the reference standard.

MATERIAL AND METHODS

Chemicals and Reagents

Isolated α glucosidase enzyme, sucrose, glucose estimation kit (Life technologies, INVITROGEN, USA). All other chemicals used for study were of analytical grade and purchased locally. A schematic presentation of the whole process in presented in figure 1.

Preparation of the aqueous extract of *Parsley* leaves (PL)

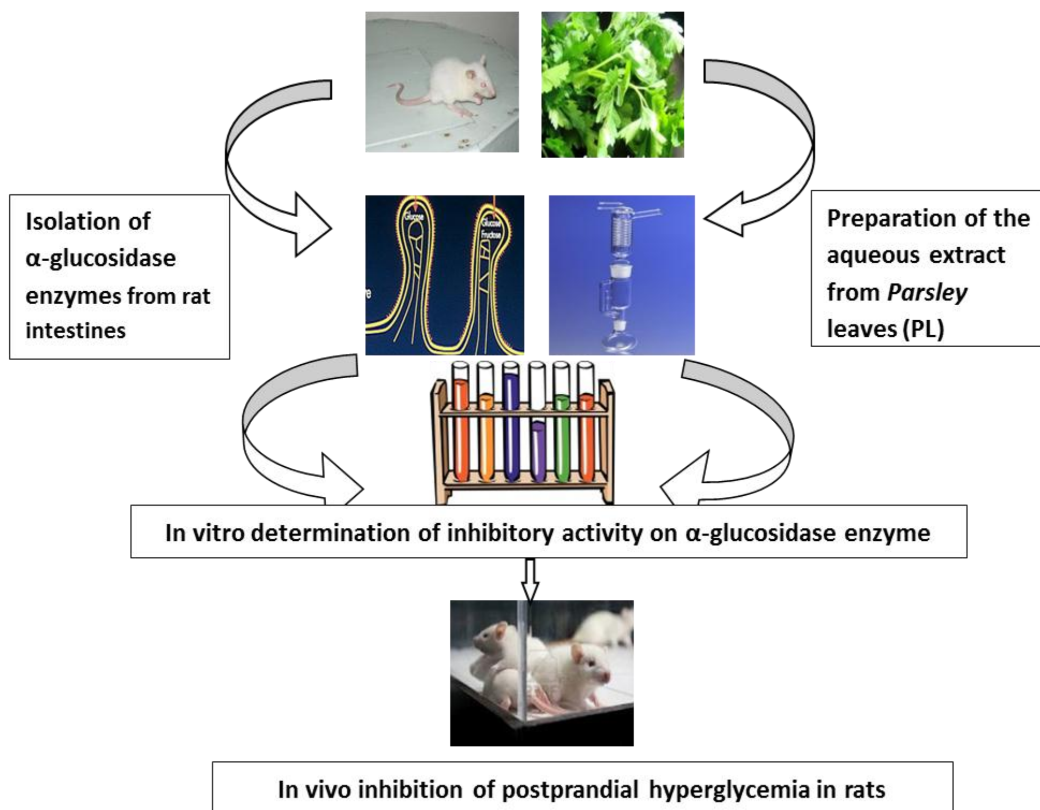
The fresh Parsley leaves were collected locally and identified by the department of pharmacognosy and photochemistry, Qassim University, Buraidah, Kingdom of Saudi Arabia. A voucher specimen is deposited for future reference. The leaves were washed well with water, shade dried for 10 days air dried and ground to a fine paste, boiled in water. Fifty grams of the paste was suspended in distilled water (500 mL) and heated to boil under reflux for 30 min. The decoction obtained was centrifuged, filtered and dried under vaccum. PL extract was prepared in distilled water on the days of experiments.

Phytochemical Screening

A preliminary phytochemical evaluation of Parsley leaves was carried to ascertain the presence of alkaloids, flavonoids, tannins, coumarins, anthraquinones, saponins, volatile oil, volatile bases, cyanogenic and sugars as per the methods described [13].

In vitro α-glucosidase inhibitory activity of PL extract

Figure 1: Schematic illustration of whole process



Isolation of α-glucosidase enzyme

All the procedure was conducted in cold conditions. A single male wistar albino rat weighing 260 gms was isolated from the animal house and fasted overnight with free access to water. The rat was sacrificed by euthanasia and a segment of the small intestine was removed, washed in 0.9% NaCl solution and chilled in ice. Intestine was cut pen and mucosa scraped off and homogenized (4000 rpm) with 4 parts of 0.1 M cold phosphate buffer (pH 6.5) for 2 hrs at 4°C. The resulting extract was centrifuged at 4000 rpm for 30 minutes. The supernatant was used for the measurement of in vitro enzyme assay. The enzyme thus obtained was used after proper dilution. One unit of enzyme activity (U) is defined as amount of enzyme forming µmole of glucose per min per ml under standard conditions. Assays were performed in triplicate using a glucose diagnosis kit based on the glucose oxidase reagent [6,7].

Determination of α-glucosidase basal activity

The basal activity of α-glucosidase enzyme was determined colorimetrically by monitoring the release of glucose from the substrate sucrose. Different dilutions of the enzyme extract (1:8, 1:4 and 1:2) with sod phosphate buffer were made and reacted with substrate and the released glucose was estimated colorimetrically to establish enzyme activity.

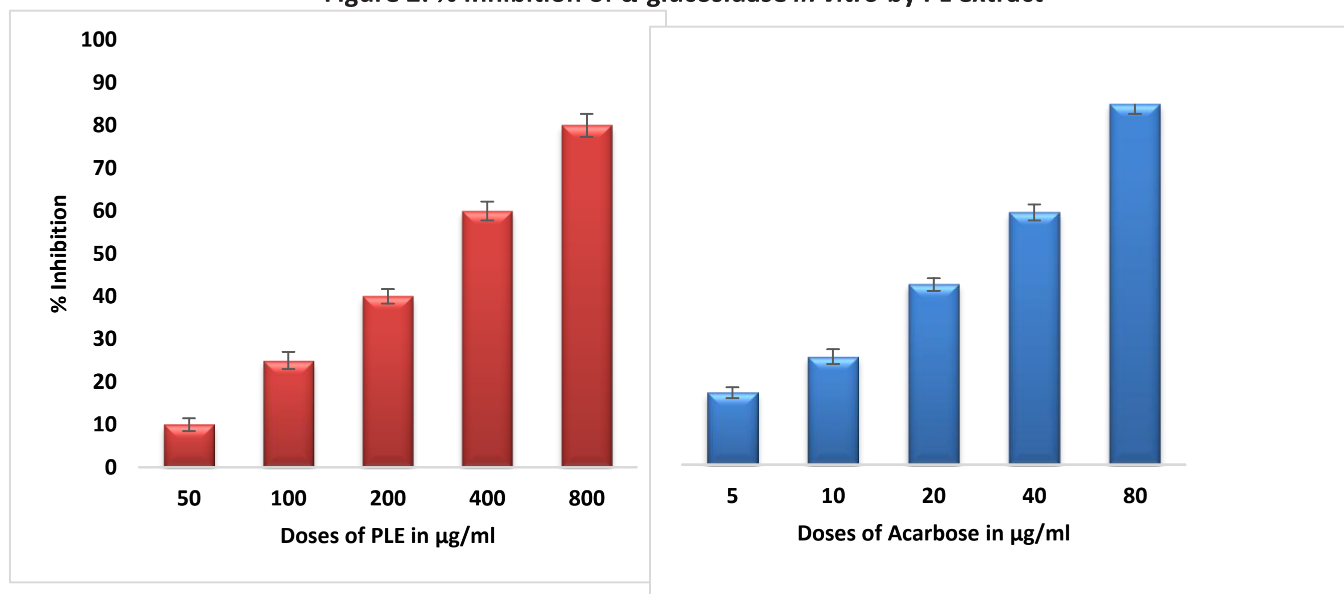
Determination of inhibitory activity of PL extract on α-glucosidase enzyme

The enzyme dilution showing highest activity (1:2) above was selected to study the inhibitory action of PL extract. The assay mixtures contained 37 nM of sucrose in mM sodium phosphate buffer (pH, 6.8) in final volume of 1ml, Twenty micro liter of PL extract (50, 100, 200, 400 and 800 µg/ml) and acarbose (5, 10, 20, 40, 80 µg/ml) in separate test tubes. The mixture was incubated for 30 min at 37 C. The reaction was terminated by boiling the solutions. The absorbance was measured and inhibitory activity was calculated as per the formula [13].

Inhibition rate (%) =
{1-(OD sample-OD blank)/ OD control} ×100

Where, OD sample is the absorbance of the experimental sample, OD blank is the absorbance of the blank and OD control is the absorbance of the control.

Figure 2: % Inhibition of α-glucosidase *in vitro* by PL extract



All the data are expressed as mean± SEM of triplicate values. PLE-Parsley Leaf Extract

In vivo inhibition of post prandial hyperglycemia by PL extract

Wistar rats of either sex weighing 200-250 g were used. They were housed in cages (six animals per cage) and kept in controlled conditions of temperature and humidity with a photoperiod of 12-h light and 12-h darkness. Pelleted food and water were available to the rats ad libitum until the start of the experiments. Rats were divided into 5 groups of six animals each: group I, control; groupS II, III, IV treated with PL extract (200, 400 and 800 mg/kg, PO); group V, treated with acarbose (800 µg/kg, PO). All the animals received sucrose (2.5 g/kg, PO) following an overnight fast whereas groups II, III, IV and V received a respective prior treatment 30 min before sucrose administration. Blood samples were taken from the tail vein at 0, 30, 60, 90, 120 and 180 min after the sucrose load. Serum was isolated from the blood samples for glucose estimation.

Statistical Analysis

All the data were expressed as mean±SEM., for experiment. Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Readings were considered significant at $p < 0.05$.

RESULTS

In vitro inhibition of intestinal α-glucosidase by PL extract

From the basal activity studies of the isolated enzyme, the dilution showing the maximum activity on substrate, in terms of glucose release, was selected for inhibitory studies (data not shown). Inhibition of intestinal α-glucosidase by different concentrations of PL extract is shown in figure 2. IC50 value for PL extract was found to be 110 µg/ml and for acarbose it was 2.5 µg/ml. PL extract produced a dose dependent inhibition. Maximum effect was to the tune of 80% whereas for acarbose it was 100%.

In vivo inhibition of postprandial hyperglycemia in rats by PL extract

The in vivo inhibition of postprandial hyperglycemia was demonstrated by administration of oral sucrose load in rats which could be due to α glucosidase inhibition. From the data in table 1 it can be observed that PL extract exhibited a dose dependent prevention in the blood glucose rise in sucrose loaded rats at interval of 60 mins when compared to control. However a maximum reduction in glucose levels was shown by 400 and 800 doses of PLE at intervals of 120 and 180 mins. This effect of PLE indicates that it has a potential in preventing postprandial hyperglycemia. On the other hand, acarbose treated rats showed a time dependent prevention in the blood glucose rise confirming the published reports.

DISCUSSION

The use of α glucosidase inhibitor in managing postprandial glucose rise is a desirable option as it can provide a comfortable means for controlling hyperglycemia. Besides this the advantage of such drugs in prevention as well as controlling the risk of

Table 1: Effect of PL extract on postprandial hyperglycemia in sucrose loaded rats

Serum glucose level (mg/dl)					
Time interval (min)	Group I Control	Group II PLE 200 mg/ml	Group III PLE 400 mg/ml	Group IV PLE 800 mg/ml	Group V Acarbose 80 µg /ml
0	64±1.3	67±2.2	63±5.2	71±1.5	69±3.8
30	165±3.5	160±3.6	159±1.8 ns	144±4.6	147±1.1
60	180±3.3	150±1.3	132±4.2*	125±6.6*	115±3.9**
120	160±3.9	123±1.5*	120±4.3*	100±4.9**	105±4.6**
180	130±5.1	110±5.2	110±5.2*	85±2.4**	81±5.9**

All the data are expressed as mean± SEM. Data are analyzed by one way analysis followed by Tukey's test. *$p<0.05$, **$p<0.01$ vs. control at corresponding time intervals, ns statistically non significant, PLE-Parsley leaf extract.

cardiovascular complications cannot be undermined especially in diabetic patients [1, 2]. In addition to these diabetic patients generally suffer from hyperglycemic shoot up after meals which require almost 4-5 hours to reduce back to the original glucose level. Such a shoot up is attributed to increased disaccharidases and glucose transporters, GLUT2 and SGLT1, activity [8, 9]. Apart from these the increased glucose level for prolonged time leads to nonspecific gyration of proteins initiating a cascade of secondary complications [3]. Hence, control of postprandial glucose levels would provide multitude of benefits to the diabetic patients.

Parsley leaves are routinely used as a vegetable and a garnishing agent as it is a part of every household [5]. A multitude of mechanisms may be involved in preventing intestinal glucose absorption like an increased gastric motility by the high fiber content of some plants may lead to reduction in intestinal glucose absorption and not necessarily due to inhibition of the sachchridase enzyme. Hence to make sure that reduction in blood

glucose in rats in the present study was due to inhibition of α glucosidase, in vitro enzyme inhibition was also carried where the extract demonstrated a dose dependent inhibition of isolated rat α glucosidase enzyme. The dose range of the extract employed was 50, 100, 200, 400 and 800 µg/ml. The extract appears to be very good inhibitor of glucosidase enzyme with a maximum of 80% inhibition with 800 µg/ml. Though acarbose appears to be a better inhibitor than PL extract in inhibitory activity but PL extract can be consumed in the regular diet, it is safe and economical, hence has a better advantage over acarbose in management of diabetes mellitus. In sucrose loaded rats the PL extract exhibited dose dependent antihyperglycemic activity with a significant effect observed post 1 hr of treatment. This property of the extract could be very useful especially in preventing post prandial hyperglycemia in diabetic patients and can help in avoiding the development of diabetes related complications.

CONCLUSION

In conclusion parsley leaves, which are commonly used and found everywhere with absolutely no reported toxicity, possess excellent antihyperglycemic effect and this may be mediated by α glucosidase inhibition. Thus these leaves can offer a more reliable and potential alternative to acarbose which is currently the most widely used α glucosidase inhibitor for diabetes mellitus. These results suggest that PL extract can generate a better lead molecule as α glucosidase inhibitor for treatment as well as prevention of diabetes mellitus particularly in control of postprandial hyperglycemia. However detailed studies are required to further confirm the claim which are underway in our lab.

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