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In Vitro Anti Diabetic Activity of Camel Milk Whey Fraction

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Abstract

The aim of this research is to determine the in vitro anti diabetic activity of camel milk whey fraction. Whey fraction was isolated from the whole camel milk according to the method described by Moradian et al., 2014. Glucose uptake capacity by yeast cells was determined according to the standard method described by Cirillo et al, 1963. Determination of alpha amylase and alpha glucosidase were carried out according to the standard methods described by McCue and Shetty., 2004 and kin et al., 2005 respectively. The results obtained were under two groups of different volumes 50µl and 100 µl. Glucose uptake under 50 µl was 88-50 ± 0.87 and at 100 µl 92.97 ± 0.19. Alpha amylase at 50 µl was 90.30 ± 0.35 and at 100 µl was 95.10 ± 0.12 Alpha glucosidase at 50 µl was 85.1 ± 0.61 and at 100 µl was 87.3 ± 0.92 camel milk post – prandial blood glucose because of its ability to enhance glucose uptake and strong inhibitory effect of both alpha amylases and alpha glucosidase activities which are known to delay the digestion of carbohydrate and hence reduce the blood glucose level.

Keywords: In Vitro, Anti Diabetic, Activity, Camel Milk, Whey Fraction

Introduction

In diabetes mellitus both carbohydrate and lipid metabolism are greatly altered due to defects in insulin production or action. It is a chronic metabolic disease that involves high health care cost and mortality rate [Lordan et al., 2013].

Recent estimate indicates there were 171 million peoples with diabetes in the world in the year 2000 and this is projected to increase to 366 million by the year 2030. The prevalence is higher in urban (10.8%) then rural (7.2%) areas and high (10.4%) than low-income countries (4%). Diabetes is associated with reduced life expectancy, significant morbidity due to specific diabetes related micro vascular complication, increased risk of macro vascular complications (ischemic heart disease, stroke and peripheral vascular disease) and diminished quality of life. The American Diabetes Association (ADA) estimated the national costs of diabetes in USA for 2002 to be 132 billion us dollars increasing to 327 billion us dollars in 2020 [Patel et al., 2012]. Plants and milk from animal sources are considered to be a source for the most potent hypo-glycemic properties [Patel et al., 2012]. Drugs from both plants and milk sources are usually non-toxic with lesser or no side effect when compared with synthetic drugs. Traditional plants that possess hypo-glycemic activity can provide useful source for discovering new compound with therapeutic value for drug development against the most common prevalent disease, diabetes mellitus [Arumugam et a[., 2013]. The total population of camel in the world is believed to be 20.89 million of which 89% are non-humped dromedary camels (camelus dromedaries) and the remaining 11% are the 2-humped (camelus bactrianus) that are generally found in the cold desserts of Asia while more than 60% of dromedary camel population is concentrated in the arid areas of North East African countries like Somalia, Sudan, Ethiopia and Kenya. Ethiopia ranks after Somalia and Sudan. Camel milk so called white gold of desert is more similar to human milk than any other milk and differs from other ruminants milk because it contains low cholesterol, low sugar, high mineral (sodium, potassium iron, copper, zinc and magnesium), high vitamin C, protective protein like lactoferrin, Lactoperoxidase immunoglobulins, lysozyme [Al-haj, 2020]. Camel milk has been acknowledged for a long time to provide a potential treatment for several diseases such as dropsy, jaundice, antihypertensive, asthma and leishmaniasis.

MATERIALS AND METHOD

Determination of glucose uptake capacity by yeast cells.

This assay was performed according to the standard method of [Cirillo, 1963]

Commercial baker's yeast was dissolved in distilled H_2O to prepare 1% suspension. The suspension was kept overnight at room temperature (25 ^oC).

On the next day, yeast cell suspension was centrifuged at 4200 rpm for 5 minutes. The process was repeated by the addition of distilled water to the pallet until a clear supernatant-fluids were mixed with 90 parts of distilled water to get a 10% v/v suspension of the yeast cells.

About 2ml v/v of camel milk whey fraction was mixed with dimethyl sulfoxide (DMSO). The mixture was then supplemented with various concentration of 1ml of

glucose solution and incubated for 10 min at 37 ºC to initiate the reaction, 100µl of yeast suspension was poured in the mixture of glucose and whey fraction vortexed and incubated for another 60 minutes at 37 ºC. After incubation, the tubes were centrifuged for 5 minutes at 3800rpm and glucose was estimated by using a spectrophotometer at 520 nm. Absorbance for the respective control was recorded on the same wavelength. The percentage increase in uptake was calculated by the formula:

% increase in glucose uptake = Abs of control-Abs Standard x 100

Abs of control

1 Determination of α- amylase inhibitory effect

the α- amylase inhibitory effect of the whey fraction was carried out using a modified method of [McCue and

Shetty, 2004]. Briefly a 250 µl aliquot of the whey fraction at different volumes were placed in a tube and 250µ1 of 0.02M sodium phosphate buffer (PH 6.9) containing α-amylase solution was added. The solution was pre-incubated at

25 ºC for 10min after which 250µ1 of 1% starch solution in 0.02M sodium phosphate buffer was added at time interval of 10 sec. then further incubated at 25 ºC

for 10 mins. The reaction was terminated after incubation by adding 1ml of dinitrosalicylic acid (DSS) reagent. The tube was allowed to boil for 10 min. and cooled to room temperature. The reaction mixture was diluted with 5ml distilled water and the absorbance was measured at 540 nm. A control was prepared using the same procedure. The result of α-amylase assay was expressed according to the following formula

$$\% \text{ inhibition} = \frac{\text{Abs of control - Abs of sample x 100}}{\text{Abs control}}$$

Determination of α- glucosidase inhibitory effect

The inhibitory effect of the whey fraction on α- glucosidase activity was determined according to the method described by [Kim et al., 2005]. The substrate solution 0.5mM p. nitrophenyl glucopyranoside was prepared in 20mM phosphate buffer, PH 6.9. An aliquot of 500µl of α- glucosidase was pre-incubated with 25 µl of different volume of camel milk whey fraction and acarbose for 10min. Thereafter, a 250 µl of 0.5mm PNPG was added as substrate to start the reaction. The reaction mixture was incubated at 37 ºC for 30 mins. The α- glucosidase activity was determined by measuring the yellow- colored p- nitrophenol released from PNPG at 405nm. The results of the α-glucosidase assay was expressed according to the following formula:

$$\% \text{ inhibition} = \frac{\text{Abs of control – Abs of sample x100.}}{\text{Abs of control}}$$

Statistical analysis

Results were recorded as means and Standard derivation of three replicates.

RESULT

Camel milk whey fraction at different volumes were assessed for in vitro anti diabetic activity by glucose uptake assay by yeast cells. Camel milk whey fraction exhibited potent activity (88.50 ± 0.87% at 50µl and
92.97± 0.19% at 100 µl).

However, in vitro anti diabetic activity was carried out in the camel milk whey fraction by using α- amylase and α- glucosidase inhibitory assays. The whey fraction showed potential inhibitory effect on these enzymes. Acarbose a synthetic drug has the ability to inhibit α amylase and α glucosidase enzymes.

Acarbose act as a Standard for both assays.

Table 1

Group	Glucose uptake (%)	Standard (%)
50 µl	88.50 ± 0.19	80.5 ± 1.2
100 µl	92.87 ± 0.19	85. ± 1.39

Values are expressed as Mean ± Standard Error Mean. (n=3)

Table 2

Group	α-amylase (%)	Standard (%)
50 µl	90.30 ± 0,35	47.63 ± 0.9
100 µl	95. 10 ± 0.12	65.32 ± 1.2

Values are expressed as Mean ± Standard Error Mean. (n=3)

Table 3

Group	α- glucosidase (%)	Standard (%)
50 µl	85.1 ± 0.61	46.71 ± 1.1
100 µl	87.3 ± 0.92	62. 83 ± 3.4

Values are expressed as Mean ± Standard Error Mean. (n=3)

Discussion

The camel milk whey fraction was assayed for antidiabetic activity in vitro by the glucose uptake method which is lucrative parameter to assess the anti-diabetic activity under in vitro condition. The extent of glucose left after a particular time in the medium was considered to be a remarkable indicator of glucose uptake [Pulivarth et al., 2020i]. The variation in the glucose uptake might be due to the extent of the solubility of

various active constituents of whey fraction. Transporting of glucose across the cell membrane was mediated by diffusion process with the intervention of stereospecific membrane carriers [Pulivarthi et al., 2020]. The other mechanism that might involve in the glucose uptake process was enhancement of cellular glucose metabolism by the camel milk whey fraction. The availability of active agents in the camel milk whey fraction might facilitate the uptake of glucose across the cell membrane. Table 1 clearly showed that camel milk whey fraction is more capable of effective glucose uptake by enhancing glucose utilization, thereby, it can control the blood sugar level.

Diabetes mellitus is a metabolic disorder a life-threatening disease which is increasing day by day. Insulin is a key player to regulate carbohydrate, fat and protein metabolism. Insulin deficiency may affect the above important metabolisms. The enzymes alpha amylase and alpha glucosidase may be responsible for the breakdown of carbohydrates into glucose. Alpha amylase is responsible for hydrolyzing the starch, which breaks down into glucose before absorption [Chelladurai and Chinnachamy, 2018]. Inhibition of alpha amylase can lead to the reduction in post prandial hyper glycemia [Chelladurai and Chinnachamy, 2018]. The inhibition of alpha amylase and alpha glucosidase enzymatic activity leads to greater or lesser carbohydrate digestion and subsequently decreasing the availability of glucose in the blood stream and resulting in the reduction in postprandial glucose in the diabetic patients [Panwar et al., 2014; ADA, 2021 and Cho et al., 2018].

From the results shown in table 2 camel milk whey fraction effectively inhibits the action of both alpha amylase and alpha glucosidase enzymes by a none dose dependent manner.

Conclusion

Camel milk whey fraction is a traditional drink popularly known in its whole camel milk form that human consumes in combination with other food to supplement diet. It is popularly used to treat diseases like cancer, ulcer and asthma. The present study established that the whey fraction isolated from the whole milk can enhance glucose absorption and inhibit alpha amylase and alpha glucosidase activities, hence may have the ability to lower post – prandial glucose level.

Recommendation

i. To determine pharmacological study in order to produce a new drug with broad spectrum activity.
ii. Camel should be integrated into the diary sector.

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