Antiglycation property of *passiflora edulis* f. Flavicarpa deg. foliage in type 2 diabetic patients

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ARTICLE INFO

ABSTRACT

Plants from the genus *Passiflora* have been chronicled in folk medicine. The leaf extract of *Passiflora edulis* has established its antiglycation property *in vitro*. Therefore this study aims to calibrate the extend of hypoglycemic property of *P. edulis* leaf extract and further provides a suitable alternative for prevention and control of hyperglycemic complications. A pre-post interventional community study was conducted in 41 type 2 DM patients by monitoring the fasting blood sugar level before and after administration of *Passiflora edulis* leaves extract for a period of 1 month. The mean plasma glucose level before the intervention was 193.44 mg/dl and during the intervention, it was reduced to 121.71 mg/dl that indicated a reduction of 37.1%. The paired 't' test using the mean values showed a strong evidence (t = 11.775, p < 0.001) for a significantly high reduction in the sugar level. The study indicates that the intake of *P. edulis* leaf extract suggests a remarkable reduction in blood sugar level during *a* four-week period in type 2 diabetic patients.

1. Introduction

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial condition characterized by mild to severe deregulation of glucose homeostasis. Exhaustive research is needed for novel methods to both prevent and treat this epidemic. Although obesity and sedentary lifestyle are known to be major risk factors for type 2 diabetes (T2DM), recent evidence suggests that oxidative stress may contribute to the pathogenesis of T2DM by either increasing insulin resistance or impairing insulin secretion.[1] Among the various species of herbal medicines *Passiflora edulis f. flavicarpa Degener* has been used extensively in the traditional system of therapeutics and now become an official drug in pharmacopeia of several countries.[2] The leaf extract of *P. edulis* plays a major role in neutralizing free radicals in the organism and thereby preventing diabetes mellitus. It also possess anxiolytic and sedative activity, as well as anti-hypertensive, anti-inflammatory, cytotoxic, antibacterial, and antifungal activity.[3]

The chemical composition of *P. edulis* presents alkaloids,[4] favanoids, sapponins[5] and mainly polyphenols.[6] A large number of studies has recently demonstrated that polyphenols have antioxidant properties and may play a major role in the prevention of various patho physiological processes associated with oxidative stress. Poly phenols may act as an antioxidant by scavenging reactive oxygen species.[7] Besides acting as antioxidants, phenols and flavonoids also inhibit amylase, sucrose as well as sodium glucose transporter-1 (S-GLUT-1) of intestinal brush border cells and hence reduces the absorption of glucose.[8] *P.edulis*. They also possess soluble fibre which significantly accelerates insulin response, an effect that was associated with an increase in postprandial active amounts of the glucose-dependent insulin tropic peptide incretin hormone (GIP) further more it reduces glucose intolerance in diabetic patients by preventing the absorption of glucose derived products from the diet.[9] The oral administration of *P. edulis* was safe up to 2000 mg/kg, it is neither hepatotoxic nor nephrotoxic.[10] This work shreds some light on the antiglycatory effect of *P. edulis* leaves in type 2 diabetic patients.

2. Methodology

The study was approved by the Institutional ethics committee as per letter number IEC/KAS/2015/10. It was a pre-post interventional design or quasi experimental design using fresh leaves of *P. edulis* collected from the south Malabar region of Kerala. Specimen identification and authentication was done as per the protocol from Regional Agricultural Research Station, Kerala Agricultural University, Palakkad (Dt.), Kerala.

The quasi-experimental research design is a comparison of outcome

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before and after a planned intervention without the use of a control group. The blood sugar measurements were taken before and after the intervention. The study thus examines whether participants in an intervention improve or become worse off during the course of the intervention, and then attributes any such improvement or deterioration to the intervention. The patients were selected through stratified random sampling technique, the strata being the age group. An age group of 35–45 years were taken as the suitable range for the selection of subjects. Only those patients with uncontrolled diabetes mellitus even after the administration of a single OHA or insulin were included in the study. The study excluded patients considered unfit during the interview and/or physical examination, which showed changes in laboratory findings; liver and renal dysfunction, severe cardiac changes and alcoholism. Throughout the study, 28 subjects were using glimepride, 26 were using metformin, 14 were on insulin followed by saxagliptine (7), glimenclamide (6), sitagliptin (3), pioglitazone (2), glipizide (2), voglibose (1), tenegliptine (1) and two subjects were not using any medications. Sample size calculation was done on the basis of generalized estimating equation approach which has been widely used to model correlated data. From January 2016 to June 2016, a total of 45 patients were evaluated, 28 women and 17 men, aged between 35 and 65 years, irrespective of ethnicity. Of those, 41 volunteers remained at the end of the experiment, 26 females and 15 males. People who did not remain until the end abandoned the study due to the bitter taste of the leaf extract. Each volunteer served as his/her own control. The study protocol followed three different stages and each one was carried out by monitoring the fasting blood glucose.

The first stage (pre-interventional period), summarized fasting blood glucose values (FBS) of the patients studied for a period of one month prior to the investigation of the yellow passion fruit leaves extract. During this period, the patients were only using medications. Various data of the patients involving demographic details, diabetic history, details of checking blood glucose, lifestyle habits, and medications were also collected using a well-prepared data collection form. The second stage involved the interventional period, where the patients were administered with the leaf extract. During this phase, oral hypoglycemic agents were discontinued and was administered only with the leaf extract. 1 L of leaf extracted water has to be consumed for 4 weeks and 3 alternative days of a week. This was justified in the subjects who were selected for the study, as they were not suitably controlled for their blood glucose levels on administration of single hypoglycemic agents. On the aspect of uncontrolled DM with single hypoglycemic agents, the need for a novel therapy which can properly control the blood glucose is considered inevitable. The subjects were informed about the potential risks involved in the discontinuation of oral hypoglycemic agents and prior informed consent was obtained accordingly. Periodical follow up was done in order to assess the changes in blood glucose levels. The third stage, was the post-interventional period, in which fasting blood glucose levels were monitored in order to verify if the glucose level would return to baseline values or not. The blood glucose levels were monitored several times during this study period and an average value was taken for the data analysis. A quasi-experimental study design can offer important insights into the care of the recruited patients and can lead to more generalizable study results based on more representative patient populations.

Blood samples from patients were collected in the morning for laboratory testing after 12 h fasting and the blood glucose levels were monitored at respective laboratories. Four blood collections were carried out during the following stages; before intake (once), during intake (twice) and after intake (once). Anthropometric indicators; height, weight and body mass index were recorded. BMI was calculated by dividing weight (kg) by squared height (m2), using the BMI ranges, adopted for nutritional classification according to the World Health Organization. Data collected for the study was tabulated in Microsoft excel 2010. By taking the rate of prevalence as 12%, with a confidence of 95% and an error of estimate of 10% the minimum sample size

worked out for the study was 41. Statistical tests used for drawing conclusion were, Paired 't' test for comparison of sugar level before and after the intervention, one factor ANOVA technique for comparison of BMI (vs.) sugar level and Chi-square test for testing the goodness of fit ratios.

3. Results

In the study, a total of 41 patients were enrolled for receiving the intervention and the mean age of diabetic patients was found to be 45 years (range: 35–65 year). Patients were categorized into three age groups; 35–45, 45–55 and 55–65 years. Chi-square test showed that there is a statistically significant difference (p value < 0.001) between the age groups ($\chi^2 = 13.480$, d. f = 2). Number of patients belonging to the age group 55–65 years are significantly higher than that of the other groups. Among the 41 patients who took an intervention, number of males was 12 (29.26%) and females were 29 (70.73%). Chi-square test showed that females are significantly ($p < 0.001$) higher in number than males ($\chi^2 = 7.048$, d. f = 1). Evaluating discordant diseases and cardiovascular diseases showed 78.04% (n = 32) had one or more chronic co-morbid disease of any type and only 21.95% (n = 9) of the patients do not have chronic co-morbid conditions. Cardiovascular diseases were the most prevalent co morbid disease at the time of diabetic diagnosis. Hypertension and hyperlipidemia are the significantly higher ($p < 0.001$) co-morbidities present in diabetes patients when compared to others ($\chi^2 = 46.667$, d. f = 7) (Table 1).

Diabetic subjects were categorized according to their BMI into normal & overweight (Table 2). Number of normal subjects was 20 (48.70%) and overweighed patients were 21 (51.21%). No significant difference ($p > 0.05$) in the number of patients could be detected in the 4 classes of overweight patients ($\chi^2 = 3.6$, d.f = 3, $p > 0.05$) as well as normal patients. The mean sugar level of overweighed patients, n = 17 (41.46%) was 193.59 mg/dl, obese patients, n = 3 (7.31%) was 206 mg/dl and normal patients, n = 21 (51.21%) was 191.21 mg/dl. The test statistic is the F value of 0.185. Since the test statistic is much lower than the critical value, we accept the null hypothesis that there is no influence of BMI on sugar level on each population and concluded that there is no significant difference in the population means. The p-value for 0.185 is greater than 0.05. So the least statistic is insignificant at that level.

Metformin and glimepiride are the most frequently used oral blood glucose-lowering medicine among type II DM patients followed by insulin, glibenclamide, saxagliptin and sitagliptin. Chi-square test showed that, significantly higher number of patients ($p < 0.001$) consuming metformin and glimepiride ($\chi^2 = 103.176$, d. f = 9). The study subjects were categorized into 5 classes according to the frequency of monitoring blood sugar level. There is a significant difference in the number of patients in the five classes ($\chi^2 = 25.707$, d.f = 4). Number of patients checking blood sugar level occasionally and one or more/week is significantly higher than the rest of the categories ($p < 0.001$).

Average levels fasting blood glucose for all patients (n = 41) before

Table 1
Distribution of study population according to morbidities.

Comorbidity	Number of patients	Percentage	Chi-square test	Degree of freedom	P-value
Hypertension	16	39.02%	46.667	7	P < 0.001
Hyperlipidaemia	16	39.02%			
Hypothyrodism	4	9.75%			
Cardiac Problems	2	4.87%			
Asthma	5	12.19%			
UTI	1	2.43%			
Arthritis	3	7.31%			
Allergy	1	2.4%			
No co-morbidities	9	21.95%			

Table 2
Dietary modification among the study subjects.

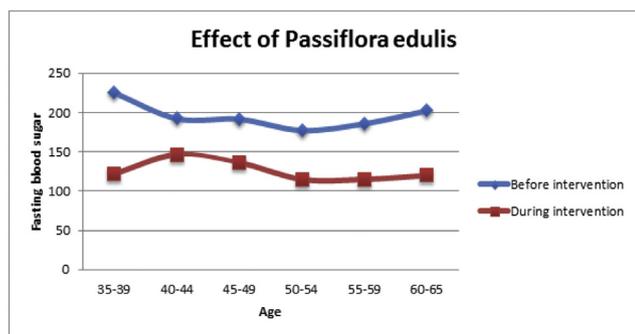| BMI	Type					Chi-square test	Degree of freedom	p-value
	Exercise (no.of patients)	Diet (no.of patients)	Exercise & diet (no.of patients)	No Exercise & diet (no.of patients)	Total (no.of patients)			
Normal	2	9	7	3	20	3.6	3	p > 0.05
	10	45	35	15				
Overweight	2	7	4	7	21	6.28		
	9.52	33.33	19.04	33.33				

Fig. 1. Antiglycation potential of *P. edulis* foliage in study subject.

and during the intervention period (30 days) shows that there was a significant difference between them. The mean plasma glucose level before the intervention was 193.44 mg/dl and during the intervention, it is reduced to 121.71 mg/dl. This showed a reduction of 37.1% in fasting blood glucose level. The paired „t" test using the mean values before and during intervention showed a strong evidence (t = 11.775, p < 0.001) for a significantly high reduction in the sugar level(Fig. 1).

4. Discussions

The treatment of diabetes mellitus and its complications is costly, and a majority of populace often seek less expensive alternatives, such as medicinal plants. In the present study, an effort has been made to find out an alternative treatment method in an attempt to reduce blood glucose level. The leaves, stems, roots and fruits of *Passiflora* species have long been used in folk medicine.[3] The leaf extract of *P. edulis* leaves has shown to possess anxiolytic and sedative activity,[11] as well as treatment for diabetes and hypertension.[12] Most individuals in this study were unable to keep their blood glucose levels in the range considered normal, even using drug therapy. We analyzed the fasting blood glucose levels two times before the intervention which showed an average of 193 mg/dl; confirming that these patients actually were uncompensated and justifying the importance of using an alternative treatment method for reducing blood glucose level. On an average reduction of 71.73 mg/dl has been observed in the total study subjects (n = 41). The Paired 't' test for comparison of sugar level before and during the intervention showed 37.1% reduction which proves the significance of the study. De Queiroz et al.[13] observed that consumption of yellow passion fruit leaf extract in patients with type II DM is benefited by a reduction in blood sugar level by a significant increase in HOMA-beta also thereby preventing diabetic complications. Although, larger and longer trials are needed to confirm these results and elucidate the mechanism involved. Evidence-based literature is enough to encourage increased consumption of foods rich in dietary fibre. The antioxidant activity of *Passiflora edulis* foliage is the core property which makes the study significant and necessitates further research on this topic. The mechanism involved in the reduction of blood sugar level is the inhibition of oxidative stress in diabetic patients by free radical scavenging process. The association between type II diabetes

mellitus and oxidative stress has been discussed in many literature. Sandra Maria el. al,[14] suggests that the genus Passiflora contains a high amount of substances in dispersible to human nutrition in the form of fibres, vitamins, mineral salts, phenolic compounds and flavonoids. According to Martina et al.,*P. edulis* leaf extracts are rich in polyphenols, especially, C-glycosyl derivatives of apigenin and luteolin such as vitexin, isovitexin, orientin and isoorientin, which are responsible for inhibition of oxidative stress. Velliyurkanniappan et al.[8] concluded that phytochemical screening of *P. edulis* leaves indicated the presence of phenols and flavonoids. The presence of these phytochemicals is responsible for the observed hypoglycemic activity of *P. edulis*. Increased oxidative stress was reported in patients with diabetes mellitus. This increased oxidative stress as a result of increased free radical formation has its contribution in vascular damage that occur in diabetes. There is a high correlation between antioxidant activity and phenolic compounds. Aliya et al.,[15] reported that phenolic compounds are the major group of compounds that acts as primary antioxidant because of its reaction with oxygen free radicals such as hydroxyl, superoxide anion radicals and lipid peroxyl radical.

The major problem we have faced during the study was the lack of similar human studies. There were many studies available for Passiflora species but many of them belong to other variants of species rather than *P. edulis* and studies related to various other parts of the plant like stem, flower and peel favour. Among the fewer *P. edulis* leaf studies 70% were rat studies and therefore, information on side effects in human were lesser. The reduction in blood sugar level observed was significant to the level of that further studies must be conducted on the anti-diabetic activity of *P. edulis* leaves.

5. Conclusions

Oxidative stress and oxidative damage to the tissue are common endpoints of chronic diseases such as atherosclerosis, diabetes and rheumatoid arthritis. The leaf extracts of *P. edulis* possess antioxidant activity against oxidative protein damage and should be considered as new sources of natural antioxidants. From the present study, it can be concluded that the oral administration of *P. edulis* leaf extract significantly decreased the blood glucose level in hyperglycemic patients. The results showed that aqueous leaf extract had best anti-diabetic activity with a maximum percentage reduction of glucose. Further studies are needed to examine the potential use of these extracts in the prevention and control of diabetes mellitus. Passion fruit, which is widely consumed, is easy to find at accessible prices, making it potentially interesting for medicinal purposes.

6. Limitations

The study elucidated only the short term effects associated with the use of leaf extracts of *P. edulis* in diabetes mellitus patients. The long term effects of the patients were not assessed due to the short time of duration of the study. Further studies are needed to examine the potential use of these extracts in the prevention and control of diabetes mellitus. The outcome measurement was limited only to the fasting blood glucose levels, HBA1C levels were not measured due to patient

related considerations.

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