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RESEARCH ARTICLE

SOY PROTEIN ISOLATE – AN ANTIDIABETIC

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ABSTRACT

Soy beans are naturally high in a group of compound known as isoflavonic phytoestrogens or isoflavones and thus help protect our body from diseases like diabetes. The objective of supplementing soy protein isolate containing high levels of isoflavones to a group of Type II diabetic subjects is to study the impact of soy isoflavones in the management of diabetes mellitus. A standardised beverage with 25g of isoflavone rich soy protein isolate mixed with water and milk was assessed for its acceptability using a nine point hedonic scale. The drink prepared with hot water without sugar was supplemented to a group of diabetic subjects for a period of six months. Physiological symptoms experienced by the diabetic group disappeared whereas no change was observed in the control group. A gradual reduction was observed in fasting blood sugar, random blood sugar, post prandial blood sugar and glycosylated haemoglobin and is statistically significant. No significant changes was observed in the control group.

INTRODUCTION

Health is considered as a fundamental human right and a world wide social goal. Degenerative diseases occur due to malfunctioning of some organ or organ systems in the body. It is certainly important to eat food which contain numerous substances with positive effects on our body and health. Soy beans are naturally high in a group of compound known as isoflavonic phytoestrogens or isoflavones (Dwyer *et al*., 1994). These plant based nonsteroidal estrogens have certain important biological activities such as antioxidant property, phytoestrogenic effect as they fit estrogen receptor sites. Soy isoflavones, plant based estrogens, help protect our body from diseases like diabetes (www.livestrong.com, 2011). They are associated with the cholesterol lowering of soy (Anderson *et al*., 1995, Anthony *et al*., 1997, Potter *et al*., 1998) to have antioxidant property in vitro (Kapiotis *et al*., 1997, Arora *et al*., 1998) and as phytoestrogens. The number of those with the global prevalence of type II diabetes has increased six fold over the past few decades, and the International Diabetes Federation calculates that the number of those with the disease will soar from 285 million in 2010 to 438 million in 2030 (IDF, 2010). Long term feeding of soy has been reported to have both hypoglycemic and hypolipidemic effects in rat (Kim, 2000). Since very little information regarding the consumption of soy protein isolate containing high levels of isoflavones in the management of diabetes mellitus are available on Indian population it was thought of interest to undertake this study with the following objectives:

Objectives

- Analyse the nutrients present in Soy Protein Isolate (SPI) containing high levels of isoflavones.
- Supplement SPI containing high levels of isoflavones to Type II diabetic subjects.
- Study the impact of supplementation of SPI containing high levels of isoflavones on the biochemical picture of diabetic subjects.

Experimental procedure

The aim of the study was to investigate upon the impact of soy isoflavones in the management of diabetes mellitus and so a group of Type II diabetics formed the subjects of the investigation.

Non Insulin Dependent Diabetic (NIDDM) subjects

One hundred NIDDM subjects of the age group of 40 to 60 years and who regularly visited the diabetic centres within a radius of five kilometers in Coimbatore city were selected at random and oriented regarding the study. Among the NIDDM subjects, 53 subjects were males and 47 were females. The severity of the condition and their willingness to participate in the six month feeding trial formed the basis of selection of 80 mild to moderate NIDDM subjects for the supplementation study. A pre tested questionnaire was formulated to elicit the socio economic background and dietary intake of the subjects. Personal interview was carried out to elicit the background information. Details included age, sex, occupation, educational status and family background. Questions regarding the dietary pattern and food intake pattern, health status like general health, diabetic trait in the family, physiological symptoms etc were also part of the questionnaire.

Nutrient analysis of SPI

SPI containing high levels of isoflavones was analysed for its nutrient content using standard procedures (NIN, 1983). The

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nutrients analysed included moisture, protein, fat, ash, calcium, phosphorus, potassium and sodium.

Standardization of isoflavone rich SPI beverage

The US Food and Drug Administration (1999) claims that "Daily consumption of 25g of soy protein as a part of diet low in saturated fat and cholesterol reduced the risk of coronary heart disease". Thus 25 g of soy protein presumably can be consumed per day safely. Therefore in this study this level was used for supplementation. As the subjects of the study preferred a drink a beverage using 25 g of isoflavone rich SPI with water was standardized. The standardized drink was given to the concerned doctors for assessing its acceptability using a nine point hedonic rating scale. For the diabetic subjects the drink was prepared with hot water and given without sugar in order to control their calorie intake.

Conduct of supplementation study

Twenty five grams of SPI containing high levels of isoflavones was supplemented to the diabetic subjects in the form of a beverage. Of the eighty subjects selected from the diabetic group, 40 subjects were given the SPI containing high levels of isoflavones mixed in hot water without addition of sugar on dialy basis for a period of six months. Of the remaining 40, 20 were given and oral drug as per diabetologists prescription remaining 20 formed the unsupplemented control group.

Assessment of nutritional status of the subjects

Anthropometric measurements

Anthropometric measurements such as height (cm), weight (kg) and BMI were assessed prior to and after supplementation.

Weight

Weight is the simplest measure of growth and nutritional status. It also provides a crude evaluation of overall fat and muscle stores. The body weight was taken using a portable weighing balance. The subjects were weighed barefooted and the weight recorded upto an accuracy of 0.1 kg.

Height

Height is a measure of linear growth of the body. For recording heights, non flexible tape was fixed on the wall and the subjects were made to stand on a flat floor with feet parallel and back of the head and heel touching the wall. The head was held erectly with arms hanging on the sides. A wooden scale was gently lowered crushing the hair and making contact with the top of the head and height was measured to 0.1 cm accuracy.

Body Mass Index (BMI)

The BMI accounts for differences body composition by defining the level of adiposity according to the relationship of weight to height, thus eliminating dependence of frame size (Stensland and Margolis, 1990). The BMI was calculated from the recorded height and weight of the subjects using the formula given by Jelliffe and Jelliffe (1989). From the formula the subjects were graded as normal, under weight, overweight and obese. BMI = weight (kg) / height (m^2)

Clinical assessment

The diabetic subjects were assessed for their physiological symptoms like polyuria, polyphagia, polydypsia, fatigue etc. before and after supplementation period.

Diet survey

A three days food weighment study was conducted among the subjects in order to quantify the actual food intake. The quantity of raw ingredients used for cooking and amount of cooked food consumed by the individual at each meal was measured for a period of three consecutive days. The raw equivalents and nutrient intake were calculated using the ICMR food composition tables (Gopalan *et al*., 1989).

Biochemical assessment

Biochemical tests are the most objective and sensitive measures of nutritional status. The subjects selected for the supplementation study were subjected to biochemical tests, relevant to their physiological status like fasting blood sugar, post prandial sugar and random blood sugar, glycosylated haemoglobin and lipid profile - total cholesterol, triglycerides, HDL, LDL and VLDL cholesterol were analysed initially after three months and finally over the supplementation period of six months.

RESULTS AND DISCUSSION

Nutrient content of the soy protein isolate

The nutrients present in the SPI containing high levels of isoflavones were analysed using the standard procedures (NIN, 1983). The moisture content of the SPI containing high levels of isoflavones is 4.7 per cent. It had a maximum of 82 per cent protein along with other minerals: calcium (3.1 per cent), phosphorus (2.3 per cent), sodium (0.75 per cent) and potassium (0.8 per cent). The fat content of the soy protein isolate was 4.2 per cent. Since soy protein isolate contained very minimal amount of fat high percentage of protein, it can safely be consumed by the diabetic subjects. The isoflavone content of the SPI is greater than 0.34 per cent. In the present study, 25 g of SPI containing greater than 85 mg of isoflavones was supplemented daily to the diabetics and pre and post menopausal women for a period of six months. In the present study 25 g of SPI with water was supplemented to NIDDM subjects for a period of six months. It contains 20.5 g of protein and 1.05 g of fat. Minerals like calcium, phosphorus, sodium and potassium present in 25 g of supplement are 0.77 mg, 0.57 mg, 0.18 mg and 0.2 mg respectively. These amounts meet 34.16 per cent and 5.25 per cent of RDA among males and 41.0 and 5 per cent among females for protein and fat respectively.

Acceptabilityof the SPI beverage

A nine point hedonic rating test ranging from "like extremely" to "dislike extremely" was used to score and thereby find out the acceptability of the SPI beverage used for supplementation. Hedonic rates relate to pleasurable or

unpleasurable experiences and are used to measure the acceptability of the food product. The SPI beverage was evaluated by the medical practitioners and by selected subjects and the mean scores obtained are presented in Table II. The first impression of any product is usually visual and a major part of our willingness to accept a food depends on its appearance. It is composite of all information about the product and its environment which reaches the eye (Birch *et al*., 1977). The appearance and colour of the SPI beverage was 7.85 and 8.15 respectively. The texture had a higher score of 8.3 for the attribute and was very smooth in texture. Due to the slight beany flavor of the beverage, the product scored only 5.35. Taste, the tactile sensation of the mouth scored 5.75. The overall acceptability score for the sensory quality characteristics of the SPI was 35.4. Though such a score does not make the beverage extremely acceptable, it is notable that the panel gave an overall average acceptability score of 8.6 which came under the category of "like very much" and hence the subjects had no apprehensives in consuming it daily. Added to this was the conviction giving to them through direct contact by giving the phytochemical properties of the beverage which is recommended for.

Effect of Supplementation of SPI

Socio economic details of the NIDDM subjects

Majority of the diabetic subjects (33) were in the age group of 41-50 years and 29 were in the age group of 57-60 years. Economic status of all the groups studied indicated that 39 subjects belonged to social class and 31 belonged to high income class and 10 were below the poverty line. Singh *et al.* (1998) has opined that social class people are significantly associated with diabetes and in this study also majority belonged to the social class. Majority of the diabetics (42) were non vegetarians, 27 were vegetarians and 11 were ova vegetarians. Consumption of both tea and coffee was by 33 subjects with coffee to 16 and only 5 with milk consumption. About 65 subjects consumed wheat and ragi based items as special food items followed by green leafy vegetables. Very few (15) subjects consumed raw vegetables and 13 consumed fenugreek. Majority of the subjects did not add sugar for the beverages and 52 avoided roots and tubers. Oil was avoided by 48 of the subjects. High intake of fat is associated with insulin sensitivity and an increased risk of developing diabetes, independent of obesity (Marshall *et al*., 1997).

43 subjects exercised regularly and 18 of them did not perform any exercise at all. Helmrich *et al.* (1991) reported that lack of physical activity has been constantly and independently related to the development of Type II diabetes. Only 18 subjects did yoga regular regularly for a period of half an hour or one hour a day. It was observed that majority of them (66 and 73) were non smokers and non alcoholic. Out of 80 subjects selected, it was found that 36 of the subjects has diagnosed the diabetic condition within a period of two years, 21 had diagnosed the condition during the past 2 to 4 years and 23 subjects within the past 6 years. 66 subjects had previous family history indicating that they could have had inherited the disease either from parents or grand parents. This observation is in line with the statement of Goldstein *et al.* (1995) that having a family history, obesity and female sex are some of the risk factors for developing diabetes. Among the selected diabetics, majority (59) were under allopathic treatment followed by homeopathy (7), naturopathy and a few ayurvedha.

Food and nutrient intake

Consumption of cereals was less than the recommended intakes by 38.2 and 25.2 per cent among the males and females respectively. Consumption of pulses followed the same trend with 32.5 and 42.5 per cent among males and females. The intake of green leafy vegetables, roots and tubers was deficit among the NIDDM subjects. The consumption of other vegetables was in excess among the males by eight per cent and females by four per cent. The intake of fruits, milk and milk products was generally high among both men (3%) and women (5%). The intake of sugar and jiggery was deficient by 75 % as the diabetics avoided them. The consumption of meat, fish and poultry, fats and oils was also deficient (23 to 26 per cent, 40 per cent).

Mean nutrient intake

The calorie intake of the NIDDM subjects was 28.1 and 7.8 per cent less than the RDA in males and females. There was 6.7 per cent deficit among males and 6.8 per cent excess among females in the intake of protein. The fat intake was very much high compared to the RDA to the tune of 25 to 71 per cent among males and females respectively. The calcium intake was excess of RDA among males (0.25 per cent) and females (14.5 per cent). The iron intake was severely deficient among the NIDDM subjects (males 53 and females 46.5 per cent). The carotene intake was markedly deficient among females in the range of 51 per cent and among males 20.5 per cent. There was an excess intake of vitamin C (males 32.5 and females 85 per cent). There was a marked deficit in the intake of fibre (80 per cent). The B vitamins namely thiamine was more pronounced among females compared to the intake of males. A balance was maintained in the intake of riboflavin among males whereas there was an excess intake of 18 per cent among the female NIDDM subjects.

Impact of supplementation of SPI on NIDDM subjects

Body Mass Index

The body mass index of the NIDDM subjects selected for the supplementation study is given in Table III. Before supplementation, 48 per cent of the NIDDM subjects fell in the obese category with BMI in the range of > 23. The remaining 26 and 6 subjects were overweight and normal with BMI in the range of 21 to 23 and 19 to 21 respectively. Adult weight gain, the degree of obesity (Pisunner, 1993) and duration of obesity are all independent and *et al*., 1996). In the present sample majority were obese and only a few were of normal weight range indicating the need to control and maintain sugar levels so as to reduce risk of diabetic complications. But after supplementation, there was a shift in BMI i.e. among the 48 obese subjects many (35) move ot the overweight and a few (7) remaining to the normal category. Whereas all the overweight subjects moved to normal BMI category. This shows that apart from the consumption of high isoflavone beverage daily, the subjects were maintaining their calorie intake and exercising regularly as per the advice of the doctor and counseling of the investigator.

Physiological symptoms

The physiological symptoms experienced by the diabetic subjects before and after supplementation for a period of six months are given in the Table IV.

Out of the 13 clinic al parameters indicative of diabetes studied nocturia, insomnia and giddiness was found in 38 subjects before supplementation and was cured completely after supplementation with the soy protein isolate beverage. Similar changes was observed in case of subjects suffering from polydypsia (27), polyuria (33), polyphagia (35), burning sensation (31), shivering (29), constipation (27) that were totally eradicated at the end of supplementation period. Excessive sweating seen among the 28 subjects at the initial stage of the study was completely absent after the supplementation period. The physiological symptoms experienced by the tablet group also showed similar decreasing trend after a six month period except for impaired vision. Whereas all the symptoms experienced by the control group remained the same.

Biochemical picture

Fasting blood sugar

The mean fasting blood sugar before supplementation in the soy supplemented and tablet group was 162.65 mg/dl and 161.9 mg/dl respectively, which was well above the normal range of 60-110 mg/dl (Trinder *et al*., 1969). A gradual reduction in the fasting blood sugar level was observed in the tablet group and the level came down to 152.43 mg/dl in three months period. A minimal change of 160.83 mg/dl was only seen in the soy supplemented group after three months. A reduction in the fasting blood sugar levels to 137.8 mg/dl and 139.6 mg/dl respectively in the soy supplemented and tablet groups was observed after a period of six months of supplementation. This reduction in the fasting blood sugar levels in the soy supplemented and tablet groups were significant at one per cent level. No such significance was observed in the control group instead there was slight (3.6 mg/dl) increase in the fasting blood sugar level. While the difference in the fasting blood sugar levels between the supplemented groups and control group were individually significant at one per cent level. This clearly indicate that over a period of time soy supplement was equally effective in bringing down the fasting blood sugar levels as the tablet and with longer periods of time probably help maintain the levels under control.

Random blood sugar

Table V gives the random blood sugar level in diabetics. Both the soy and tablet groups showed a significant ($P<0.01$) effect in decreasing the random blood sugar of the diabetic subjects. The mean random blood sugar levels before supplementation was 233.24 mg/dl, 231.65 mg/dl and 224.20 mg/dl in the SPI, tablet and control groups respectively which were much higher than the normal range of 80-140 mg/dl (Trinder *et al*., 1989). There was a maximum reduction in the tablet group after a period of six months and the random level was brought down to 186.95 mg/dl compared to the soy supplemented group which had a level of 206.15 mg/dl of random blood sugar. No such significant reduction was seen in the control group. When compared between groups, all values were significant at one per cent level. From the above results, it appears that the tablet was comparatively more effective than the soy protein isolate in bringing down the random blood sugar levels. But the fact that isoflavone rich soy protein brought about a gradual reduction is indicative of its potential to reduce random blood sugar levels over a period of time and may be longer periods of supplementation can give conclusive evidence for normal maintenance.

Post prandial blood sugar

The mean post prandial blood sugar levels before supplementation was 257.85 mg/dl in the soy supplemented group, 258.27 mg/dl in the tablet group and 251.45 mg/dl in the control group which was much higher than the normal range of 60-140 mg/dl (Trinder *et al*., 1989). After a period of three months, a reduction of 253.86 mg/dl and 244.24 mg/dl was observed in the soy supplemented and tablet groups respectively. There was also a reduction due to supplementation with soy protein isolate and tablet over a period of six months. It is notable that the post prandial blood sugar level in the SPI group came down to 239.3 mg/dl with the mean difference of 18.55 mg/dl over a period of six months whereas in the tablet group it came down to 223.97 mg/dl with a mean difference of 34.3 mg/dl. But, in both the supplemented groups the level did not come down to the normal range and longer time of supplementation probably can bring it down further to normal range. The difference in the control group was negligible. This reduction in the supplemented groups was significant at one per cent level. Comparisons of the mean post prandial blood sugar level between the three groups were significant at one per cent level. Comparison of post prandial blood sugar levels between the soy protein isolate supplemented and tablet groups show that tablet was more effective in reducing the post prandial blood sugar level than the SPI group during the supplementation period of six months. However, the trend of soy supplement containing high isoflavone in lowering the post prandial blood sugar levels gradually is a promising indication that longer period of supplementation could probably bring down the value of normal levels. Food supplement being less harmful to human system and probably the most cost effective and sustainable strategy, such an effect of isoflavones rich soy have promising implications and needs long term supplementation studies (Figure 2).

Glycosylated haemoglobin

The mean blood glycosylated haemoglobin levels of the diabetics in the soy supplemented group (8.8 %/100 ml), tablet group (8.7 %/100 ml) and control group (8.61 %/100 ml) initially were above the non-diabetic level (4-5.6 %/100 ml) (Trivelli *et al*., 1978) and placed all of them in the poor control group. A reduction to 8.1 %/100 ml was evident in the tablet group but no change was observed in the soy supplemented group and control group after a period of three months. But after a period of six months there was a slight decrease from the initial value of 8.8 %/100 ml to 8.2 %/100 ml in the soy supplemented group and a decrease to 7.8 %/100 ml was observed in tablet supplemented group. The decrease in both the supplemented groups were statistically significant ($P<0.01$). No change was observed in the control. Tablet supplemented group gave a more significant ($P<0.01$)

Table I. Proximate principle composition of the soy protein isolate

Nutrients	In 100 g of SPI	In 25 g of SPI	RDA (males)	Percent intake	RDA (females)	Percent intake
Moisture (g)	4.17	0.18	-	-	-	-
Protein (g)	82	20.5	60	34.16	50	41.0
Fat (g)	4.2	1.05	20	5.25	20	5.25
Calcium (mg)	3.1	0.77	400	0.19	400	0.19
Phosphorus (mg)	2.3	0.57	800	0.07	800	0.07
Sodium (mg)	0.75	0.18	-	-	-	-
Potassium (mg)	0.8	0.2	-	-	-	-
Isofalvones (mg)*	340	85	-	-	-	-

* As per the values by Protein along proterotein Technologies India Limited

Table II. Acceptability scores of the spi beverage

Criteria	Score
Appearance	7.85
Colour	8.15
Texture	8.3
Flavor	5.35
Taste	5.75
Overall acceptability	35.45

Table III. BMI of the niddm subjects

N=80

BMI	Before supplementation	After supplementation
19-21 Normal	6	39
21-23 Overweight	26	35
>23 Obese	48	6

Table IV. Physiological symptoms

Physiological symptoms	Groups					
	SPI		Tablet		Control	
	Initial	Final	Initial	Final	Initial	Final
Polydypsia	27	-	14	2	12	12
Polyuria	33	-	18	1	19	19
Polyphagia	35	-	17	-	14	14
Nocturia	38	3	18	5	18	18
Excessive sweating	28	-	12	-	9	9
Burning sensation during micturition	31	2	8	-	10	10
Constipation	27	-	13	-	15	15
Insomnia	38	2	17	-	19	19
Shivering	29	-	11	-	12	12
Giddiness	38	-	19	-	18	18
Impaired vision	8	6	3	3	3	3
Hesitancy during micturition	26	-	8	-	11	11

Table V. Changes in random blood sugar levels

Groups	Random blood sugar (mg/dl)					
	Initial mean±SD	After 3 months	Final mean±SD	Mean difference	't' value	Between groups
SPI	233.25	229.28	207.15	-27.1	23.03**	S Vs T
(N=40)	±30.80	±25.42	±29.34	±7.4		8.21**
Tablet	231.65	208.32	186.95	-44.7	23.35**	S Vs C
(N=20)	±23.09	±19.97	±22.26	±8.56		15.03**
Control	224.2	224.09	223.1	-1.1	1.73NS	T Vs C
(N=20)	±27.12	±27.13	±27.24	±2.84		21.62**

**Significant at one per cent level NS Not significant

decrease than the soy protein isolate supplemented group (Figure 3). These results are in line with the observation of Kim (2009). From the foregoing parameters on sugar levels of the diabetics on sugar levels of the diabetics on supplementation with soy protein isolate containing high levels of isoflavones it could be clearly seen that the blood glucose levels and the glycosylated haemoglobin levels came down more or less as effectively as the tablet supplemented group. However, long term supplementation may help further reduce the blood glucose levels and maintain them under control. Further long term supplementation studies are required to give conclusive evidence.

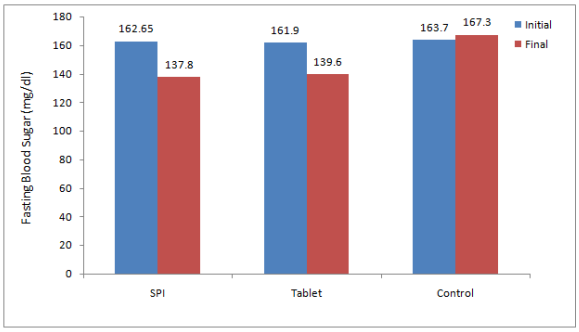

Figure 1. Changes in fasting blood sugar levels of the niddm subjects

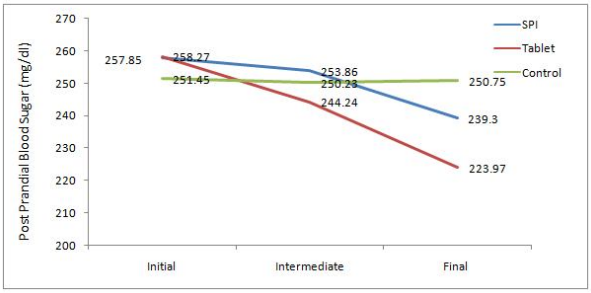

Figure 2. Changes in post prandial blood sugar levels

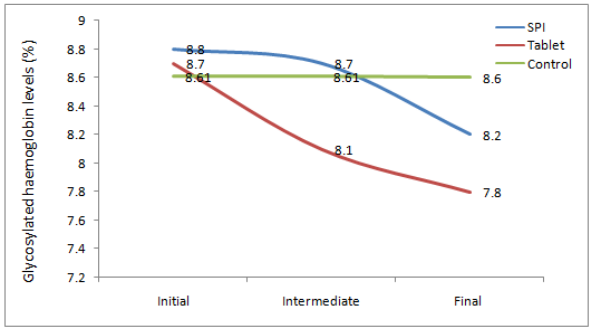

Figure 3. Changes in glycosylated haemoglobin

Conclusion

This study though limited in number of sample and period of supplementation beyond doubt proves the presumable beneficial impact of soy rich in isoflavones in the management of Type II diabetes. Being a dietary source, rich in both micro and macro nutrients including trace elements like zinc and selenium and fibre, it is needless to further emphasize the miraculous effect of soy isoflavones in daily dietaries. If soy isoflavones in the form of soy protein isolate can become the daily adjunct of dietaries the benefits it can give to humans suffering from degenerative disease with or without the ageing proves is commendable.

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