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IMPACT OF HYPOGLYCEMIC HERBAL MIXTURE BASED BISCUITS INTERVENTION ON BLOOD GLUCOSE LEVEL AND LIPID PROFILE OF TYPE 2 DIABETIC SUBJECTS

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ABSTRACT

Diabetes mellitus is a growing health concern worldwide and the prevalence of diabetes is projected to rise from 285 million in 2010 to 438 million in 2030. Several herbal plants possess hypoglycemic properties, and to avail their hypoglycemic effect, these are used as medicine by diabetics. Herbal plants have rarely been incorporated in food preparations. Keeping this in view, hypoglycemic herbal mixture (HGHM) was prepared by using three herbal plants namely, fenugreek seeds, bitter gourd and *gurmar* leaves. Salty biscuits were developed by incorporating HGHM at 12 per cent level. Impact of feeding hypoglycemic herbal mixture based biscuits was studied in 30 type 2 male diabetics. Daily feeding intervention with four biscuits (1 g HGHM/ salty biscuit) for a period of 3 months significantly reduced the fasting as well as post prandial serum glucose. A significant improvement in the serum lipid profile by lowering serum total cholesterol, triglycerides and very low density lipoprotein- cholesterol was also observed, thus helping in retarding the secondary complications of the disease.

Key Words: bitter gourd, fenugreek seeds, *gurmar* leaves, type 2 diabetes, serum glucose, serum lipid profile.

INTRODUCTION

Diabetes mellitus is a group of diseases characterized by high blood glucose concentrations due to defects in insulin secretion, insulin action or both (Marion, 2000). Long standing diabetes mellitus causes chronic complications including microvascular, macrovascular and neuropathic disorders. Worldwide, the prevalence of diabetes is projected to rise from 285 million people in 2010 (representing 6.6% world population) to 438 million people in 2030, corresponding to 7.8% of the adult population (Colagiuri, 2010). According to World Health Organization (WHO), approximately 31.7 million people in the year 2000 were diabetic in India and it is estimated that by 2030, the number would be more than double. The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and less physical activity (Wild et al., 2004).

Oral hypoglycemic drugs are the mainstay of treatment of diabetes but these have prominent side-effects, lag behind poor patient compliance and are too expensive. In comparison to conventional drugs, medicinal plants & herbs possess no or fewer side effects, are easily available and have relatively low cost (Valiathan, 1998; Venkatesh et al., 2003). Herbs refer to not only the herbaceous plant but also to bark, roots, leaves, seeds,

flowers and fruits of trees, shrubs and woody vines. Herbal medicine is based on the premise that plants contain natural substances that can promote and alleviate illness. More than 1200 plant species have been recommended for the treatment of diabetes (Jouad et al., 2000; Eddouks et al., 2003). World Health Organization (WHO) has also substantiated the utilization of herbal remedies for the management of diabetes (Bailey and Day, 1989). In relation to diabetes and its complications, medicinal plants, namely, *Trigonella foenum graecum*, *Gymnema sylvestre*, *Momordica charantia* are amongst some of the most effective and commonly studied antidiabetic medicinal plants (Grover et al., 2002; Mukherjee et al., 2006). Although various studies have proven the hypoglycemic effect of fenugreek seeds (Sharma and Raghuram, 1990), bitter gourd (Ahmed et al., 1999) and *gurmar* leaves (Baskaran et al., 1990), still people are not familiar or aware of their beneficial effect. Due to non-acquaintance of consumption form of herbal plants and non-acceptability of taste in their original form (powder form), it is difficult to continue the intake of these herbal plants for longer duration. Efforts are needed to develop some food based nutritional supplements using these herbal plants which can serve as an alternative, particularly if they are economically and socioculturally viable and acceptable. Hence, the present study was planned to access

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the effect of feeding intervention with herbal mixture based biscuits on serum glucose level and lipid profile of type 2 diabetics.

MATERIALS AND METHODS

PROCUREMENT AND PROCESSING OF HERBAL PLANTS

The raw material fenugreek seeds (*Trigonella foenum graecum*) and bitter gourd (*Momordica charantia*) were purchased in bulk from local market of Kurukshetra, whereas *gurmar* leaves (*Gymnema sylvestre*) were purchased from the cultivator of herbal plants in Kurukshetra.

PROCESSING OF HERBAL PLANTS

FENUGREEK SEEDS

Fenugreek seeds were sorted and cleaned to remove impurities and grounded to fine powder in an electric grinder. The fine powder of fenugreek seeds was stored in an airtight plastic container for further use.

BITTER GOURD FRUIT

Fresh, immature bitter gourd were washed thoroughly in water to remove adhering foreign materials, surface water was removed by spreading on filter paper sheets followed by drying in hot air oven at $45 \pm 5^{\circ}\text{C}$ till complete drying. The dried bitter gourd was ground to fine powder and stored in an airtight plastic container till further use.

GURMAR LEAVES

The *gurmar* leaves were separated from the stalk, cleaned of foreign materials, washed in clean water and surface water was removed by spreading on filter paper sheets. Thereafter, *gurmar* leaves were dried in hot air oven at $45 \pm 5^{\circ}\text{C}$ till complete drying. The dried leaves were ground to fine powder and stored in an air tight plastic container for further use.

PREPARATION OF HGHM

Hypoglycemic herbal mixture was prepared by mixing powdered form of *gurmar* leaves, bitter gourd fruit and fenugreek seeds in equal proportion (1:1:1).

DEVELOPMENT OF HYPOGLYCEMIC HERBAL MIXTURE BASED BISCUITS

In the departmental food laboratory, hypoglycemic herbal mixture based biscuits (HGHMBB) were developed by incorporating HGHM at different levels (ranged from 10-15 %) in salty biscuits. The organoleptic evaluation was done by a panel of twelve judges (comprising of 8 faculty members of Department of Food and Nutrition and 4 diabetic people) using nine point Hedonic scale (Amerine et al., 1965). On the basis of their judgement and composition, most acceptable level i.e. 12

per cent (1 g of HGHM/ biscuit) was selected for supplementation (Table 1).

Table 1: Composition of Hypoglycemic Herbal Mixture Based Biscuits

| Ingredients | Amount |
|---------------------------------------|----------|
| Whole Wheat Flour | 500 g |
| Refined oil | 150 ml |
| Sugar free (powder) | 15 g |
| Powdered hypoglycemic herbal mixture* | 120 g |
| Ammonia powder | 12-15 g |
| Milk | 150 ml |
| Salt | to taste |

**Gurmar* leaves: Fenugreek Seeds: Bitter Gourd:: 1:1:1

SELECTION AND FEEDING OF THE SUBJECTS

A total of 30 type 2 diabetic male subjects between the age of 40-60 years were selected by purposive selection technique. Subjects were recruited from Kurukshetra University Health Centre, Kurukshetra. Individuals with chronic complications of diabetes, cardiovascular diseases, chronic alcohol and drug abuse were excluded from the study. The study was approved by the Institutional Ethical Review Committee of Kurukshetra University, Kurukshetra and informed consent was obtained from the subjects. After collecting and analyzing blood for glucose and lipid profile of selected 30 type 2 diabetic subjects, were followed for one and a half month. During this period, these subjects were taking their routine diet along with prescribed medicine which they were already taking on the recommendation of their doctor. This period was treated as self control period. After one and a half month, fasting and postprandial blood samples were again collected and analyzed. This day was taken as 0th day of feeding trial and feeding trial with hypoglycemic herbal mixture was commenced.

PRE-TESTING OF HYPOGLYCEMIC HERBAL MIXTURE BASED BISCUITS

Before commencing the supplements feeding intervention, hypoglycemic herbal mixture based biscuits were given to five diabetic patients for ten days for testing physiological functioning of gastrointestinal (abdominal pain, flatulence, gastric upset) and no side effect and drug interaction was observed among subjects.

FEEDING OF HYPOGLYCEMIC HERBAL MIXTURE BASED BISCUITS

Each subject was fed 4 salty biscuits (2 salty biscuits ½ hour before breakfast and 2 salty biscuits ½ hour before dinner) for a period of 90 days. The HGHM based salty biscuits were distributed to the patients on weekly basis to ensure regular consumption of these products as per instructions given to them.

COLLECTION OF BLOOD SAMPLE

Five ml of fasting and post prandial (2 hour after meal) blood samples of selected 30 type 2 diabetics were collected in the beginning (before commencing the feeding intervention with HGHMBB), after one and a half month period (self control period). Thereafter 45 days of feeding and after 90 days of feeding period.

ANALYSIS OF BLOOD SAMPLES

Fasting serum samples were analyzed for glucose, total cholesterol, triglycerides and high density lipoprotein cholesterol using a Carelab 200 Photometer (Semiautomated blood analyzer), while low density lipoprotein cholesterol and very low density lipoprotein cholesterol were calculated from primary measurements using the empirical formula of Friedewald equation (Friedewald et al., 1972).

Serum glucose and total cholesterol were determined by GOD (glucose oxidase) /POD (Peroxidase) method of Trinder (1969) and enzymatic method of Allian et al. (1974), respectively. Serum HDL cholesterol was analyzed by precipitating method of Naito (1984) and Grove (1979). Serum triglycerides were estimated by using Autopak Reagent Kit by enzymatic method (Buccolo and David 1973; Werner et al., 1981; and Annoni et al., 1982).

STATISTICAL ANALYSIS

All statistical analysis was undertaken using the Statistical Package for Social Sciences (SPSS) version 16.0. Mean and standard deviation were calculated for each studied variable. A paired 't' test analysis was performed to assess whether significant differences existed in biochemical parameters of the subjects after end of self control period. One Way ANOVA was used for testing the variation in biochemical parameters among the subjects after end of feeding period. Level of significance was set at $P \leq 0.05$.

RESULTS AND DISCUSSION

The serum glucose level and lipid profile of diabetics initially, before and after feeding hypoglycemic herbal mixture based biscuits (HGHMBB) are summarized in Table 2. Slight decrease in mean initial values of fasting serum glucose, post prandial serum glucose, fasting serum total cholesterol, fasting serum triglyceride, fasting low density lipoprotein cholesterol and fasting very low density lipoprotein cholesterol, whereas, slight increase in HDL-cholesterol were observed after one and a half month of self control period, however the decrease was found to be non-significant.

Table 2: Mean (\pm SD) Blood Biochemical Parameters Of Male Diabetic Subjects Initially (I) On 0th Day (B), 45th Day (D) And 90th Day (A) Of Feeding Interventions Based On Hypoglycemic Herbal Mixture Based Biscuits

| Biochemical Parameters (mg/dl) | Initial Level (I) | Change in Biochemical Parameters of Blood with feeding interventions on | | | f value [#] | P |
|--------------------------------|--------------------|---|--------------------------|--------------------------|----------------------|--------|
| | | 0 th day (B) | 45 th day (D) | 90 th day (A) | | |
| Fasting serum glucose | 156.50 \pm 12.86 | 153.98 \pm 13.32 | 151.86 \pm 13.36 | 142.88 \pm 14.07 | 5.64 | .005** |
| Postprandial serum glucose | 212.60 \pm 38.60 | 204.67 \pm 34.83 | 183.84 \pm 26.89 | 148.36 \pm 14.79 | 28.17 | .000** |
| Serum Total Cholesterol | 196.88 \pm 25.64 | 196.12 \pm 21.36 | 190.98 \pm 23.16 | 182.00 \pm 20.57 | 3.21 | .045** |
| Serum Triglycerde | 168.31 \pm 25.50 | 165.93 \pm 26.38 | 157.86 \pm 25.29 | 149.56 \pm 23.54 | 3.192 | .046** |
| Serum HDL-Cholesterol | 41.40 \pm 4.90 | 41.60 \pm 5.07 | 42.32 \pm 5.14 | 43.70 \pm 5.50 | 1.237 | .295* |
| Serum LDL - Cholesterol | 121.80 \pm 23.10 | 121.33 \pm 22.19 | 117.08 \pm 24.45 | 108.45 \pm 21.85 | 2.47 | .09* |
| Serum VLDL -Cholesterol | 33.66 \pm 5.60 | 33.18 \pm 5.27 | 31.57 \pm 5.05 | 29.91 \pm 4.70 | 3.19 | .046** |

**Significant (Level of significance $P \leq 0.05$), ([#]f- value is given for feeding period of 90 days)

Non-significant difference between mean initial value (I) and 0th day of feeding intervention for all analyzed biochemical parameters of blood (Level of significance as tested by paired t-test)

The mean fasting serum glucose level of the subjects on 0th day of feeding intervention was 153.98 \pm 13.22 mg/dl and after 90 days of feeding intervention with hypoglycemic herbal mixture based biscuits (HGHMBB) it

was significantly reduced to 142.88 \pm 14.07 mg/dl. The findings of the present study are in concurrence with those reported by Baskaran et al. (1990) who affirmed that administration of GS₄, the water- soluble acidic fraction of an ethanol extract of the leaves of *Gymnema sylvestre* to type 2 diabetic patients significantly reduced the fasting blood glucose. Ahmad et al. (1999) observed that feeding of homogenized suspension of the vegetable pulp of

Momordica charantia to 100 moderate type 2 diabetic subjects caused a significant reduction in fasting glucose in 5 percent subjects.

The post prandial serum glucose of the diabetic subjects had a significant reduction (27.51 %) at day 90 (148.36 ± 14.79 mg/dl), when compared to the 0th day of intervention (204.67 ± 34.83 mg/dl). The results of present study are in agreement with the studies reported by Shanmugasundaram et al. (1990); Neeraja and Rajyalakshmi (1996); Kuppu et al. (1998), Ahmad et al. (1999) and Gupta and Lal (2001) who separately stated that *Momordica charantia*, *Gymnema sylvestre* and fenugreek seeds exhibited hypoglycemic effect. Shimizu et al. (1997) reported that extract of *Gymnema sylvestre* leaves suppressed elevation of blood glucose level by inhibiting glucose uptake in the intestine. Fenugreek seeds have been shown to lower blood glucose levels and partially restore the activities of key enzymes of carbohydrate and lipid metabolism to normal values in various animal model systems (Vats et al., 2002).

A significant decrease (7.20%) in the fasting serum total cholesterol level (14.12 mg/dl) of diabetic with hypoglycemic herbal mixture based biscuits which further reduced the risk for cardiovascular disease in diabetic patients (Table 3). Bordia & Srivastva et. al subjects was observed after 90 days of feeding intervention (1997) reported that administration of fenugreek seed to

coronary artery disease patients with type 2 diabetes mellitus significantly decreased the total cholesterol. Khanna (1998) reported that protein fraction ‘gourdin’ extracted from bitter gourd seeds reduces cholesterol level in diabetic patients and has no side effects. Presence of dietary fibre in fenugreek seeds and bitter gourd may affect serum cholesterol by reducing cholesterol and bile acid absorption by altering the metabolism and ratio of bile acid absorbed, by changing the intestinal secretion and hepatic production of lipoprotein (Chen & Aderson et al. 1986).

The serum triglycerides of the diabetic subjects had a significant reduction (9.87 %) at day 90 (149.56 ± 23.54 mg/dl), when compared to the 0th day of intervention (165.93 ± 26.38). Studies reported that diabetic state, resulting from an impaired secretion and sensitivity of insulin may be responsible for high triglycerides level in serum than normal individuals, as the insulin stimulated the synthesis of adipose tissue by agency of lipoprotein lipase (Matshushita et al. 1982). Similar decrease in triglycerides levels of the diabetics were observed by feeding fenugreek seeds (Sharma et al., 1998). The finding of the present study are also in agreement with the findings of Khanna (1998) who reported that protein fraction ‘gourdin’ extracted from bitter gourd seeds reduces triglyceride level in diabetic patients and has no side effects.

Figure 1: Mean (\pm SD) Blood Biochemical Parameters Of Male Diabetic Subjects Initially (I), On 0th Day (B), 45th Day (D) And 90th Day (A) Of Feeding Interventions Based On Hypoglycemic Herbal Mixture Based Biscuits

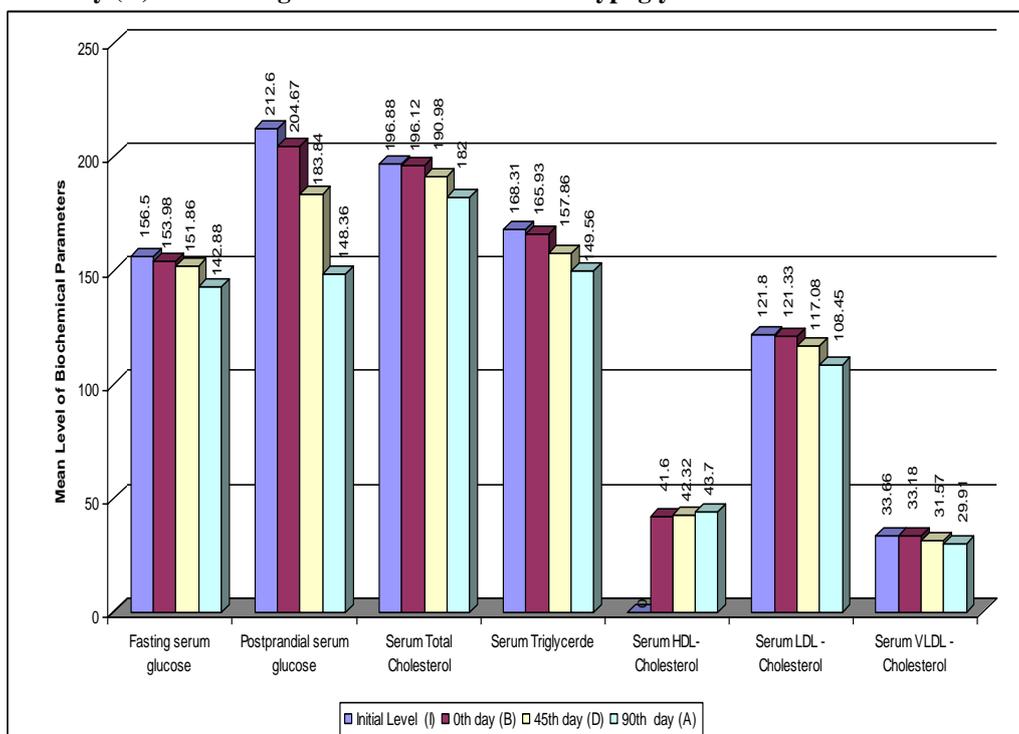


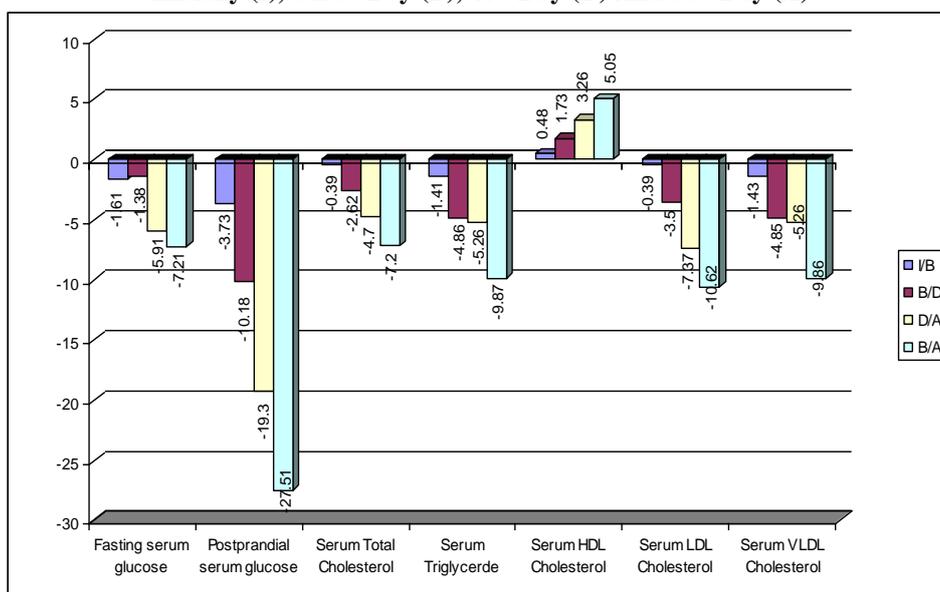
Table 3: Per Cent Change In Mean Levels Of Serum Glucose And Fasting Lipid Profile Of Male Diabetic Subjects Initially (I), On 0th Day (B), 45th Day (D) And 90th Day (A)

| Biochemical Parameters (mg/dl) | I/B | B/D | D/A | B/A |
|---------------------------------|-------|--------|--------|--------|
| Fasting Serum Glucose | -1.61 | -1.38 | -5.91 | -7.21 |
| Postprandial Serum Glucose | -3.73 | -10.18 | -19.30 | -27.51 |
| Fasting Serum Total Cholesterol | -0.39 | -2.62 | -4.70 | -7.20 |
| Fasting Serum Triglyceride | -1.41 | -4.86 | -5.26 | -9.87 |
| Fasting Serum HDL-cholesterol | 0.48 | 1.73 | 3.26 | 5.05 |
| Fasting Serum LDL-cholesterol | -0.39 | -3.50 | -7.37 | -10.62 |
| Fasting Serum VLDL-cholesterol | -1.43 | -4.85 | -5.26 | -9.86 |

On feeding with hypoglycemic herbal mixture based biscuits for 90 days, a significant reduction of 11 per cent in the VLDL-cholesterol was observed, whereas, no significant change was observed in HDL-cholesterol and LDL- cholesterol concentrations (Table 2 and 3). However, Sharma et al. (1998) reported significant

reduction in total cholesterol, low density lipoprotein cholesterol, very low density lipoprotein cholesterol and triglyceride levels after ingestion of an experimental diet containing 25 g fenugreek seed powder in 60 type 2 diabetic patients.

Figure 2: Per Cent Change In Mean Levels Of Serum Glucose And Fasting Lipid Profile Of Male Diabetic Subjects Initially (I), On 0th Day (B), 45th Day (D) And 90th Day (A)



CONCLUSION

The salty biscuits developed by incorporating hypoglycemic herbal mixture at 12 per cent level (1 g HGHM/ biscuit) were found to be quite acceptable by the diabetic subjects. Feeding intervention with four biscuits per day for 90 days showed a significant improvement in fasting serum glucose, post prandial serum glucose, fasting serum total cholesterol, fasting serum triglyceride and fasting serum very low density lipoprotein - cholesterol. Hence, it is suggested that diabetic patients should be encouraged to incorporate these herbal plants in their daily diet to control serum glucose level and lipid profile.

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