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Effect of dietary fiber in lowering serum glucose and body weight in sprague dawley rats

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Abstract

Introduction:

The present study evaluated the hypoglycemic perspectives and weight loss significance of dietary fiber. Dietary fiber was supplemented in commercial wheat flour (atta) for the preparation of chapaties, a staple diet of South Asia. Male Sprague Dawley rats (n = 100) were randomly divided into 4 diet groups (n = 25 per group). The control group was fed basal diet that included commercial wheat flour chapati, cornstarch, corn oil, salt and vitamin mixture in such a way that 10% of the protein was available from the final diet. To the basal diet of other 3 groups, chapaties supplemented with 2% guar gum (GG 2%), 3% guar gum (GG 3%) and 5% chickpea + 1% guar gum (CP5%+GG1%) were added, respectively. All diets were fed to the rats for a period of 8 weeks to perceive the impact of respective compositions. Rats fed on CP 5% + GG 1%, showed maximum glucose reduction (14.57%) followed by GG 3% (11.64%) and GG 2% (9.60%) as compared to control diet. Likewise, rats fed on 3% GG showed maximum decline (7.90%) in body weight. It was concluded that chapaties prepared from selected treatments provide an additional dietary fiber that could be supportive for diabetic and obese individuals.

Results:

The results indicated that addition of dietary fiber influenced the physical characteristics of chapati non-significantly. Maximum glucose concentration was found to be 112.50 mg/dL in control group followed by 101.70 and 99.41 mg/dL in groups fed on guar gum 2% and guar gum 3%, respectively. Lowest glucose concentration (96.11 mg/dL) was observed in rats fed on the combination of chickpea 5 %+ guar gum 1%. Maximum serum protein concentration was found to be 6.39 g/dL in rats fed on combination of chickpea 5 % + guar gum 1% whilst the remaining three groups showed non significant variations with respect to each other. Means for serum

protein were 6.33, 6.30 and 6.32 g/dL for control, guar gum 3%, and guar gum 2%, respectively. Maximum serum albumin concentration was found to be 3.63 g/dL in rats fed on combination of chickpea 5%+ guar gum 1% showing non-significant differences than that of control (3.60 g/dL).

Conclusion

Soaring cost of medication and their side effects demand new ways against the existing malady of diabetes. Diet based strategy is a right approach as it is economical and assessable to avoid the health risks. The present research explored that diet diversification is an effective tool for the management of serum glucose and body weight. Role of legumes is indispensable to enhance the dietary fiber. Ingestion of chapaties prepared from selected compositions of composite flours providing an additional dietary fiber would be supportive to reduce hyperglycemia and obesity.

Keywords: Sprague Dawley rats, Dietary fiber, Composite flour, Chapati, Serum glucose, Insulin glucose indices.

BACKGROUND:

Studies on the composite flours containing wheat and legumes have been carried out in various parts of the world to explore their therapeutic and medicinal role. Legumes are not only important to improve the nutritional status of the diet but also for the preparation of fiber enriched products. It is apparent that composite flour prepared by blending wheat and legumes in proper proportions may increase the fiber content of the diet resulting in easiest bowel movement, reduction in cholesterol level in serum and liver and beneficial effects particularly to the diabetics [1, 2]. Legumes are the second to cereals as essential sources of dietary fibers. Chickpea, pigeon pea, mung bean, urad bean, lentil and field pea are the pulse crops of significant dietary and economic importance in the sub-continent [3].

Total dietary fiber includes all food components that are not broken down by human alimentary tract enzymes to assailable fragments [4]. Dietary fiber adds no calories to the diet. It may protect against diabetes and obesity [5]. Wheat contains 12% of total fiber out of which 9-11% is insoluble while the remaining 1-3% comprised of soluble fiber [6]. In case of legumes, total dietary fiber content ranged from 10.7-14.3% [7]. According to another study, chickpea and lentil have dietary fiber content 16.3% and 15.9%, respectively [8]. Guar gum (GG) contains 75% soluble fiber and 7.6% insoluble fibers [9]. Water-soluble dietary fiber acts like a sponge and absorbs water in the intestine, mixes the food into gel and there by slows down the rate of digestion and absorption. Glycemic index of pulses and legumes have generally ranged from 20 to 50% of that of white bread [10]. Owing to the enormous functions of GG, United States (US) imports more than 96 million pounds of GG from India and Pakistan annually [11].

Cereal grains and legumes play an important role in supplying the nutrients, as well as over 70% of the daily energy requirements [12]. In Pakistan, 80% of the wheat flour produced is utilizing in the form of unleavened flat bread locally known as Chapati, a staple diet of South Asia while the rest 20% is using in bakery section. More than 60% of the total daily requirement of protein and calories of Pakistani people are met through wheat [13]. When a staple food that is

consumed regularly by the masses is used as a vehicle, high population coverage can easily be achieved [14]. Therefore, chapaties prepared by blending wheat flour with lentil, chickpea, and GG can be beneficial for the normal individuals as well as hyperglycemic patients. During in vitro degradation studies, highly significant decline in sugar release by dietary fiber enriched biscuits is shown. This has a beneficial effect in potentially reducing the glycemic index and subsequent glycemic loading of such foods [15]. In Pakistan, chickpea flour was blended with wheat flour to bake "*basini roti*" that is unleavened bread commonly consumed by diabetic patients [16]. Wheat flour can be enriched with pulses up to 10% to produce bread of acceptable quality [17]. Consumption of composite flour bread prepared with 5% guar gum and 5% wheat bran decreased the level of postprandial blood glucose when compared to the level of fasting blood glucose. The effect of guar gum on reduction in postprandial glycemia was due to delayed passage of guar gum containing meal through the stomach and small intestine, also might be due to the viscous nature of the meal resisting the propulsive and mixing effects of the gastrointestinal contractions, thereby reducing access of the glucose to the absorptive epithelium [18,19]. Guar gum can be effectively used to reduce postprandial blood glucose [20]. The acute effect of a single dose of guar gum has been verified to reduce the peak postprandial whole blood glucose levels (about 10%). Following long-term treatment, a further reduction was seen in the obese subjects with the highest postprandial glucose levels [21].

Dietary fiber certainly has some potential in the management of weight loss. This effect is derived from the potential influence of fiber on several aspects of food intake and nutrient availability [22]. The effects on weight loss are often deduced from effects on satiety, decreased caloric intake, and increased fecal excretion of energy in the form of fat and nitrogen [23-25]. It has been found that gel-forming fibers such as guar gum and pectin are more effective in promoting weight reduction than non-gel forming fibers like wheat bran [26]. Supplementing a normal diet with gel-forming fibers, such as guar gum leads to an increased satiation probably due to slow gastric emptying. A long term studied had confirmed the usefulness of viscous fibers as an adjunct to regular dietary treatment of obesity [27]. The long-term effects of guar gum in male adolescent rats were studied; the guar gum group consumed less diet throughout the entire study and gained less weight over the first 20 weeks as compared to the cellulose and bran groups [28]. Rats fed on guar gum had reduced feed intake and body weight gain [9]. Guar gum (10 and 20g/Kg in diet) can slow the mechanical disruption of food in the stomach and thereby impede the delivery of nutrients to the small intestine [29]. Modified guar gum has appeared to affect appetite and body weight (BW) loss in humans. A significant decrease in body weight (62.9 ± 2.9 vs. 60.4 ± 2.2 kg in paired comparison) in subjects receiving guar gum was observed in middle-aged females [30]. In contrast, a gain in body weight of rats fed on fortified samples of wheat with gram flour was reported [31]. Comparative diets containing zero, 50 and 100g/Kg GG were fed to male Wistar rats for 21 days. Weight gain over the balanced period and feed conversion ratio decreased linearly with increasing GG intake [32].

Raw legume feeding resulted in greater pancreatic and small intestine weight relative to body weight [33]. Diets containing 0, 10 and 20 % guar gum were fed to normal male wistar rats (107.0 ± 9.3 g) for 60 days, which showed 10 % increase in small intestine length [9]. In another study, significant increase in small intestinal length was found in guar-supplemented rats [34]. In

Pakistan, to control obesity and diabetes, little efforts are being carried out through diet diversification programs. It is the dire need to increase dietary fiber in the diet of vulnerable group by blending high dietary fiber commodities in the staple diet i.e. chapati to reduce the threat of glycemic indices and obesity. For this purpose, research trials were conducted on male Sprague Dawley rats to find out the impact of composite flour chapaties with special reference to serum glucose and gain in body weight to attain the conclusive approach.

MATERIALS AND METHODS

The research was carried out in National Institute of Food Science & Technology, University of Agriculture, Faisalabad-Pakistan.

Raw Material

Commercial wheat flour *(atta)*, guar gum (*Cyamopsis tetragonolobus*), lentil (*Lens culinaris),* and chickpea (*Cicer arietinum)* were purchased from the market to make composite flour compositions for chapati preparation.

Preparation of Composite Flours

Wheat flour was blended with lentil, chickpea and guar gum flours in different combinations as mentioned in Table 1. Each composition of composite flour was thoroughly mixed to achieve a uniform dispersion of legume flour particles with wheat flour. Three homogenous replicates for each composite flour was taken and stored at room temperature in woven polypropylene bags.

Table 1. Different combinations used to prepare composite flours.

Treatments	Wheat flour %	Lentil %	Chickpea %	Guar gum %
T_1	100	-	-	-
T_2	95	5	-	-
T_3	92.5	7.5	-	-
T_4	90	10	-	-
T_5	95	-	5	-
T_6	92.5	-	7.5	-
T_7	90	-	10	-
T_8	99	-	-	1
T_9	98	-	-	2
T_{10}	97	-	-	3
T_{11}	94	5	-	1
T_{12}	91.5	7.5	-	1
T_{13}	89	10	-	1
T_{14}	94	-	5	1

T_{15}	91.5	-	7.5	1
T_{16}	89	-	10	1

- T_1 (commercial wheat flour) acts as control

Preparation of Chapaties

Chapaties of uniform thickness (3 mm) and weight (100g) was prepared and baked at different intervals on a thermostatically controlled hot plate at a temperature of 210 °C for 2.5 minutes [35].

Dietary Fiber

Composite flours and resultant chapaties were evaluated on dry weight basis for dietary fiber at 0, 30, and 60 days interval by a combination of enzymatic and gravimetric procedures [36].

Sensory Evaluation

The Sensory evaluation of chapaties was carried out for various attributes to determine the total chapati scores at stated intervals by a trained taste panel using 9-point hedonic scale system [37].

Selection of Best Compositions

Based on the better performance of samples for product preparation regarding sensory attributes of chapaties and dietary fiber three best compositions along with control were selected. These samples were used further for efficacy purposes.

Efficacy Studies

Animal Care Committee of National Institute of Food Science and Technology, University of Agriculture, Faisalabad approved the study. Efficacy studies were conducted on four groups of male Sprague Dawley rats (n = 100), twenty-five in each group (each rat in an individual cage) to determine the effect of three best-selected compositions with that of control on serum profile with special reference to glucose, protein, and albumin. The rats were fed on freshly prepared diets (Table 2) and water on daily basis up to 8 weeks.

Table 2 Composition of experimental diets.

Ingredients	A	B	C	D
Chapati (g)	85.84	87.72	87.11	83.40
Corn Starch (g)	4.66	2.78	3.39	7.10
Corn oil (ml)	5.00	5.00	5.00	5.00
*Salt mixture (g)	3.50	3.50	3.50	3.50
**Vitamin mixture (g)	1.0	1.0	1.0	1.0
Total (g)	100	100	100	100
Protein from all sources	10.01	10.02	10.00	10.00

A = (Control diet) prepared from chapati containing 100% commercial wheat flour
B = Diet containing guar gum 3%
C = Diet containing guar gum 2%
D = Diet containing chickpea 5%+ guar gum 1%

*Salt mixture (Calcium citrate, Ca $(H_2PO_4)_2$ H_2O, H_2HPO_4, NaCl, $CaCO_3$, $3MgCO_3$. Mg $(OH)_2$. $3H_2O$, $MgSO_4$ anhydrous, Ferric ammonium citrate, $CuSO_4$. $5H_2O$, NaF, $MnSO_4$. $2H_2O$, KAl $(SO_4)_2$. $12H_2O$ and KI)
**Vitamin mixture (Thiamin hydrochloride, riboflavin, pyridoxin hydrochloride, calcium, pentothenate, nicotinic acid, inositol, p-aminobenzoic acid, biotin, folic acid, cyanocobalamin, choline chloride and maize starch)

The feed intake of individual rat was determined by deducting the leftover and spilled over diet from the total amount supplied per day. Gain in body weight of individual rat in each group was determined on weekly basis throughout the experimental period to find out the effect of individual diet on body weight. The temperature 23±2 °C and 12 hours lighting period were maintained through out the experimental period. At the end of the trial after eight weeks, the overnight fasted rats were decapitated and the blood samples were collected. Glucose concentration of individual rats in each group was calculated by GOD-PAP method [38]. Similarly, serum protein was determined by Biuret method [39] where as albumin concentration was determined by Bromcresol Green method [40]. After serum collection, the organs i.e. liver, heart, lungs, spleen, right kidney, left kidney, and small intestine were separated and weighed to determine the effect of experimental diets. The intestine length was also measured with the help of scale.

Statistical Analysis

Analysis of variance was carried out to determine the level of significance by applying completely randomized design by using Minitab® 15 Statistical Software [41].

RESULTS

Dietary Fiber: The means for dietary fiber content in composite flour samples and resultant chapaties have been shown in Table 3.

Table 3. Dietary fiber content (%) of composite flours and chapaties (Means ± standard deviation).

	Control	GG 3%	GG 2%	CP5%+GG1%	P value	Confidence Intervals
Composite flours	6.72^{d}±0.19	8.15^{b}±0.0.25	8.85^{a}±0.22	7.90^{c}±0.22	0.000	7.46-8.34
Composite flour chapaties	6.03^{c}±0.18	7.48^{b}±0.22	8.16^{a}±0.20	7.28^{bc}±0.23	0.000	6.79-7.68

Means showing similar lettering in a row were not statistically different to each at 1% level of significance
Control=Commercial wheat flour; GG3% = Guar gum 3%; GG2% = Guar gum 2%; CP+GG = Chickpea 5% + Guar gum 1%;

It is obvious from the results that variation (P<0.000) exists within various compositions of composite flours. Maximum dietary fiber 8.85% was observed in T_{10} (guar gum 3%) followed by 8.15% in T_9 (guar gum 2%) and 8.10% in T_{16} (chickpea 10% + guar gum 1%) where as least dietary fiber content (6.72%) was found in T_1 (commercial wheat flour). It is apparent from the results that variations (P<0.000) exist regarding dietary fiber in chapaties prepared from different composite flours. Maximum dietary fiber 8.16% was observed in chapaties prepared from T_{10} (guar gum 3%) followed by 7.48% in T_9 (guar gum 2%) and 7.43% in T_{16} (chickpea 10% + guar gum 1%) where as minimum dietary fiber content (6.03%) was found in chapaties prepared from

T_1 (commercial wheat flour). Moreover, T_{14} (chickpea 5%+ guar gum 1%) also exhibited a dietary fiber value of 7.28% with non significant differences with T_{16}. The values in Figure 1, illustrated that percent augmentation of dietary fiber in chapaties prepared from guar gum 3% (T_{10}) was 35.32% followed by T_9 (guar gum 2%) i.e. 24.05% and T_{14} (chickpea 5% + guar gum 1%) i.e. 20.73% as compared to control. There is a progressive increase in dietary fiber with the adding up of guar gum. The addition of guar gum not only enhanced dietary fiber but also increased one of its important fraction i.e. soluble fibers, which play a key role to control glucose, obesity and cholesterol in the body. It is suggested from the present study that ingestion of chapaties prepared from composite flours containing guar gum 3% followed by guar gum 2% and chickpea 5% + guar gum 1% provides an additional dietary fiber that would be supportive for the hyperglycemic people.

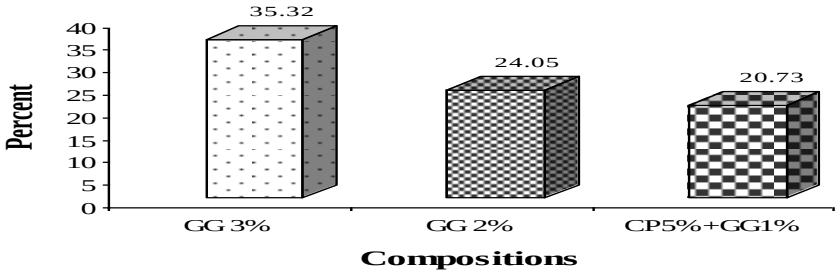

Figure 1. Percent increase in dietary fiber of chapaties prepared from different flour samples compared to control.

Sensory Evaluation: 'Chapati' is the staple diet of sub-continent; the consumer favors a chapati of light brown and creamy in color which can be smashed to pieces of the desired size and folded to form a scoop. The sensory evaluation of chapati for various sensory attributes such as color, flavor, taste, texture, foldingability, chewingability was carried out at and the overcome of all sensory attributes is presented in the form of total chapati scores. Means for total chapati scores and their sum of all sensory attributes showed (Figure 2).

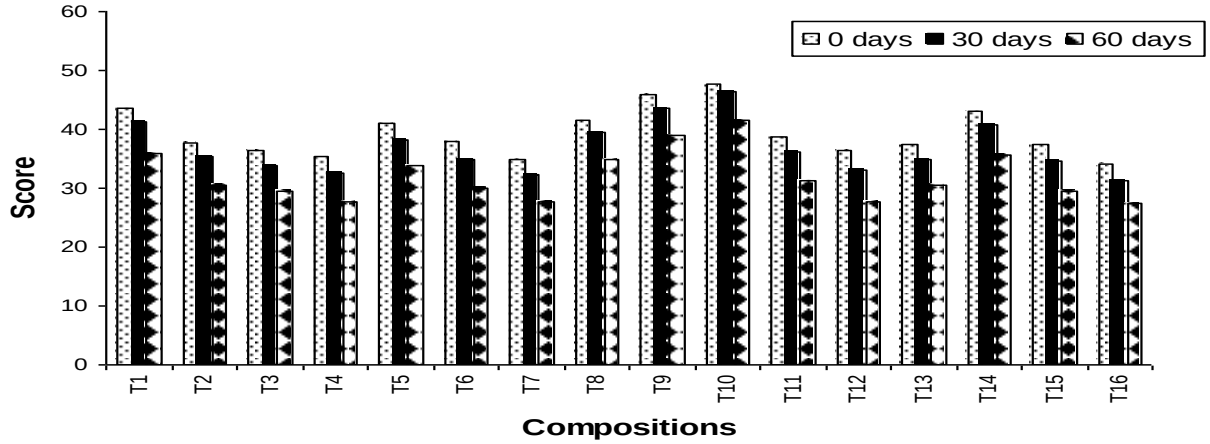

Figure 2 Total chapati score.

T_1 = Commercial wheat flour
T_2 = lentil 5%
T_3 = lentil 7.5%
T_4 = lentil 10%
T_5 = chickpea 5%
T_6 = chickpea 7.5%
T_7 = chickpea 10%
T_8 = guar gum 1%
T_9 = guar gum 2%
T_{10} = guar gum 3%
T_{11} = lentil 5%+ guar gum 1%
T_{12} = lentil 7.5%+ guar gum 1%
T_{13} = lentil 10%+ guar gum 1%
T_{14} = chickpea 5%+ guar gum 1%
T_{15} = chickpea 7.5%+ guar gum 1%

Chapaties prepared from T_{10} flour acquired the highest scores i.e. 45.27 (47.80, 46.40 and 40.60 at 0, 30 and 60 days, respectively) followed by T_9 i.e. 42.87 (46.00, 43.60, and 39.00 at 0, 30 and 60 days, respectively). Moreover, T_1 i.e. 40.27 (43.60, 41.20 and 36.00 at 0, 30 and 60 days, respectively) where as T_{16} (chickpea 10% + guar gum 1%) got the lowest total chapati scores i.e. 31.00 (34.20, 31.40 and 27.40 at 0, 30 and 60 days, respectively). It was further observed that T_{14} got 39.87 (43.20, 40.80 and 35.60 at 0, 30 and 60 days, respectively) scores with non-significant differences to T_1.

Physical Parameters: Means for feed, water intake, and gain in body weight of different groups of rats fed on different treatment have been presented in Table 4.

Table 4. Feed intake, water intake, gain in body weight and serum bio-chemical profile in different groups of rats (Means ± standard deviation).

Parameter	Control	GG 3%	GG 2%	CP5%+GG1 %	P value	Confidence Intervals
Feed intake (g/rat/day)	17.13[a] ±0.07	15.95[d] ±0.08	16.45[c] ±0.10	16.92[b] ±0.08	0.001	16.35-16.88
Water intake (mL/rat/day)	28.10 [d] ±0.18	30.48 [a] ±0.14	29.94 [b] ±0.17	29.32 [c] ±0.16	0.000	28.95-29.97
Gain in body weight (g/rat/week)	6.96 [a] ±0.16	6.41[c] ±0.19	6.55 [b] ±0.08	6.99 [a] ±0.15	0.000	6.58-6.87
Serum glucose (mg/dL)	112.50 [a]±0.74	99.41 [c]±0.81	101.70 [b]±0.83	96.11 [d]±0.69	0.000	98.88-105.97
Serum protein	6.33 [b]±0.01	6.30 [b] ±0.01	6.32 [b]±0.01	6.39 [a]±0.01	0.000	6.32-6.35

(g/dL)						
Serum albumin (g/dL)	3.60 [ab] ±0.01	3.55 [b] ±0.01	3.58 [b] ±0.01	3.63 [a] ±0.01	0.003	3.57-3.61

Means showing similar lettering in a row were not statistically different to each at 1% level of significance
Control=Commercial wheat flour; GG3% = Guar gum 3% ; GG2% = Guar gum 2% ; CP+GG = Chickpea 5% + Guar gum 1% ;

It is apparent from the results that rats fed on control diet had the highest feed intake (17.13 g/day) followed by the rats fed on diets containing chickpea 5 % + guar gum 1% (16.92 g/day) and guar gum 2% (16.45 g/day). It was also observed that the lowest feed intake was in the diet containing guar gum 3% (15.95 g/day). Figure 3 depicted percent reduction of feed intake in different groups of rats as compared to control group. It was found that guar gum 3% resulted in 6.89% decrease in feed intake followed by decrease of 3.97% and 1.23% for guar gum 2% and chickpea 5%+ guar gum 1%, respectively (P=0.001). Study period significantly affected the feed intake in rats. The results showed that highest feed intake was observed on 6th week (17.82g) followed by 8th (17.61g) week and 5th (17.56g) while the lowest feed intake was observed in 1st week (15.08g) followed by 2nd week (15.57g) per rat per day.

It is apparent from the results (Table 4) that highest water intake (30.48 ml/day) was observed in rats fed on diet containing guar gum 3% followed by (29.94 ml/day) in guar gum 2% and (29.32 ml/day) in rats consuming chickpea 5%+ guar gum 1% (P<0.000). Lowest water intake (28.10 ml/day) was observed in control (commercial wheat flour). There is a progressive increasing trend in water consumption by increasing guar gum in the diet. From percent increase of water intake in different groups of rats with reference to control (Figure. 3), it was established that guar gum 3% resulted (8.47%) increase in water intake followed by guar gum 2% (6.55%) and chickpea 5%+ guar gum 1% (4.34%) as compared to control. Water intake was not affected appreciably and all groups of rats followed a parallel trend during the entire study period**.**

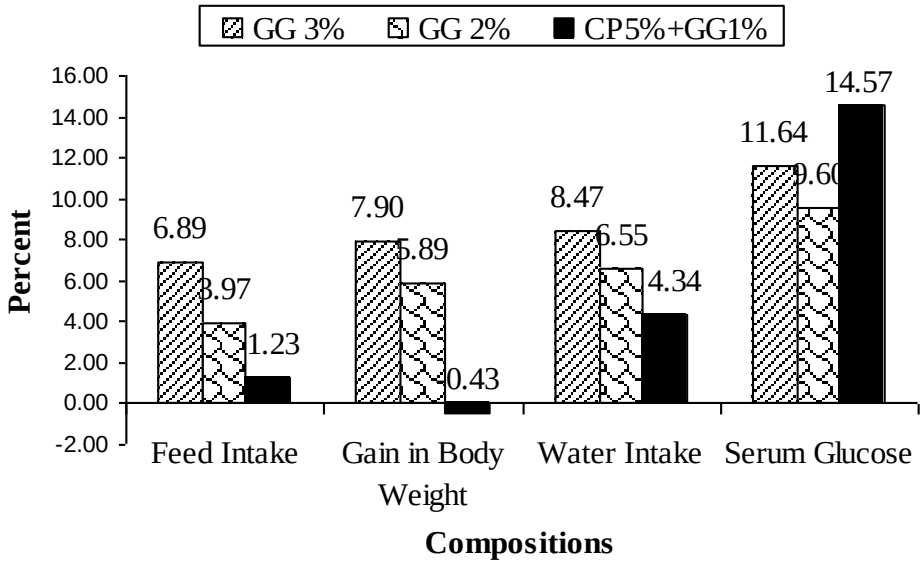

Figure 3. Percent decrease of feed intake, gain in body weight, serum glucose and increase in water intake in different groups of rats compared to control.

The body weight of the rats varied with age as well as sex. In the first sex weeks, there is the highest increase in body weight while with the passage of time, progressive decrease in gain in body weight was seen. Likewise, the male rats gain more weight than the female rats of the same age. Highest gain in body weight (P<0.000) was observed in rats fed on diets containing chickpea 5% + guar gum 1% (6.99g per rat/week) and control (6.96g per rat/week). It showed that both had non significantly different to each other followed by guar gum 2% (6.55g per rat/week) while the lowest body weight gain was noted in guar gum 3% (6.41g per rat/week) fed rats (Table 4). It was established (Figure 3) that rats fed on guar gum 3% showed highest decrease in gain in body weight 7.90% followed by 5.89% in rats fed on guar gum 2%. However non-significant differences were observed in case of chickpea 5% + guar gum 1% with reference to, control. The results with respect to study period showed that highest gain in body weight was observed in 4^{th} week (9.23g) followed by 5^{th} week (7.91g) and 2^{nd} week (7.73g) per rat per week. Similarly, an increase (4.35g) in gain in body weight was observed in 1^{st} week. After 4^{th} week, decreasing trend for gain in body weight was observed in all groups of rats but this decrease was more pronounced in rats fed on guar gum 3%. It is concluded from the present exploration that guar gum 3% followed by guar gum 2% by amalgamation in chapati is supportive for the management of weight loss and obesity.

Organs Weight: Means for organs weight of different groups of rats express that diets prepared from selected compositions of composite flours have meaningful effect on intestine weight and intestine length of rats where as liver, heart, lungs, spleen, left kidney and right kidney remained unaffected by using various diets (Table 5). Maximum intestine weight (8.06g) was observed in rats fed on guar gum 3%, followed by guar gum 2% (7.98g) where as non-significant increase was observed in control (7.68g) and chickpea 5% + guar gum 1% (7.64g) group (P=0.0365). Dietary fiber supplementation in the form of guar gum resulted in increase of intestinal length (P=000). The mean values for intestine length of different groups of rats explored that diets prepared from various compositions significantly affected intestine length. Rats fed on both guar gum 3% and guar gum 2% showed maximum intestine length i.e. 1.17 and 1.16 m, respectively. In case of chickpea 5 % + guar gum 1%, intestine length remained unaffected (1.11m) with reference to control group (1.10m). It was found that guar gum 3% resulted in maximum increase (6.36%), followed by guar gum 2% (5.45%) where as non significant difference (0.91%) was noted in rats fed on chickpea 5% + guar gum 1% in comparison with control group.

Table 5 Organ weight of different groups of rats (Means ± standard deviation).

Organ weight	Control	GG 3%	GG 2%	CP5%+GG1%	P value	Confidence intervals
Liver (g/100g)	4.25±0.07	4.21±0.05	4.22±0.05	4.20±0.05	0.9271	4.15- 4.27
Heart (g/100g)	0.574±0.01	0.532±0.01	0.536±0.01	0.548±0.01	0.1290	0.534- 0.561
Lungs (g/100g)	0.992±0.03	0.970±0.02	0.978±0.02	0.939±0.01	0.4355	0.946- 0.994
Spleen (g/100g)	0.347±0.01	0.322±0.01	0.315±0.01	0.336±0.02	0.0932	0.320- 0.340
Right kidney (g/100g)	0.529±0.01	0.513±0.01	0.508±0.01	0.493±0.01	0.0862	0.501 -0.521
Left kidney	0.566±0.01	0.547±0.01	0.548±0.01	0.546±0.01	0.7532	0.537- 0.567

(g/100g)						
Intestine (g)	7.68[b]±0.12	8.06[a]±0.13	7.98 [ab]±0.13	7.64[b]±0.10	0.365	7.71-7.96
Intestine length (m)	1.10[b]±0.01	1.17[a]±0.01	1.16[a]±0.00	1.11[b]±0.00	0.000	1.12-1.15

Means showing similar lettering in a row were not statistically different to each at 1% level of significance
Control=Commercial wheat flour; GG3% = Guar gum 3%; GG2% = Guar gum 2%; CP+GG = Chickpea 5% + Guar gum 1%;

Analysis of Serum Bio-Chemical Profile

Serum Glucose: Regarding serum glucose, It is apparent (Table 4) that there exists high variation (P<0.000) variations among different groups of rats fed on various diets of composite flours. Maximum glucose concentration was found to be 112.50 mg/dL in control group followed by 101.70 and 99.41 mg/dL in groups fed on guar gum 2% and guar gum 3%, respectively. Lowest glucose concentration (96.11 mg/dL) was observed in rats fed on the combination of chickpea 5%+ guar gum 1%. Percent decrease of glucose concentration (Figure 3) in different groups of rats with reference to control explicated that chickpea 5%+ guar gum 1% showed maximum reduction (14.57%) followed by guar gum 3% and guar gum 2% which induced 11.64% and 9.60% reduction in glucose as compared to control. The glucose level in rats also varied with age and sex. In young male rats, the glucose level ranged from 80-120 mg/dL. From the present investigation, it was concluded that there is 10-14% decline in glucose by the addition of selected compositions. Keeping in view the present findings, it is desirable to incorporate chickpea and guar gum at the mandatory levels as proved in the study in the staple diet i.e. chapati, to reduce serum glucose level of hyperglycemic persons.

Serum Protein: Significant differences regarding serum protein were observed in different groups of rats fed on various diets Table 4. Maximum serum protein concentration was found to be 6.39 g/dL in rats fed on combination of chickpea 5%+ guar gum 1% while the remaining three groups showed non significant variations with respect to each other (P<0.000). Means for serum protein were 6.33, 6.30, and 6.32 g/dL for control, guar gum 3%, and guar gum 2%, respectively. From the present investigation, it is concluded that almost all groups of rats followed the similar trend for serum protein except for chickpea 5% + guar gum 1% fed group in which there is a slight increase in the protein as contrast to control.

Serum Albumin: As obvious from Table 4, significant contribution of various compositions has been noted for albumin (P=0.003). Maximum serum albumin concentration was found to be 3.63 g/dL in rats fed on combination of chickpea 5%+ guar gum 1% showing non-significant differences than that of control (3.60 g/dL). Remaining two groups i.e. guar gum 3% and guar gum 2% showed non-significant variation with respect to each other. Means for serum albumin were 3.55 and 3.58 g/dL for guar gum 3% and guar gum 2%, respectively however; they also showed non-considerable differences with control.

DISCUSSION

Dietary Fiber: Dietary fiber may be defined as food components not broken by human alimentary tract enzymes like hemicelluloses, pectic substances, gums, mucilages, cellulose and lignin as well as undigested protein and lipid [4]. The average value for dietary fiber in wheat flour (70-80% extraction) was 3.6% while whole grain flour contains 14.4% on dry weight basis consisting of 1-3% of soluble fiber [42]. Additional daily intake of 10g fiber appeared to lower the risk of coronary death by 17% [43, 44]. In the current research, commercial wheat flour was used having dietary fiber content 6.72%. Guar gum is a rich source of dietary fiber providing approximately 85% on dry weight basis [45] that is the main reason to have a higher dietary fiber content of composite flour samples containing guar gum. In the present study, different legumes are tested in chapati with the idea to increase the dietary fiber to control blood glucose and weight gain through the staple diet of the region. Dietary fiber is inevitable for hyperglycemic individuals because 1g of soluble fiber from oats, psyllium, pectin, or guar gum produced changes in total cholesterol of -0.037, -0.028, -0.070, and -0.026 mmol/L (-1.42, -1.10, -2.69, and -1.13 mg/dL), respectively. LDL cholesterol was -0.032, -0.029, -0.055, and -0.033 mmol/L (-1.23, -1.11, -1.96, and -1.20 mg/dL), respectively [18].

Sensory Evaluation:

During the present work, conditions were identical for the preparation of chapaties however, the significant effect of legumes and their combinations showed a strong impact on total chapati scores. The decreasing trend in the total chapati scores during storage may be due to the development of rancidity, proteolytic & lipolytic activity and probably due to mold and infestation. The chapaties prepared from T_{10} (guar gum 3%), followed by T_9 (guar gum 2%) and T_{14} (chickpea 5% + guar gum 1%) along with T_1 (commercial wheat flour) got the highest total chapati scores and were preferred over the others by the judges. In the present study, different composite flour samples affected the overall chapati quality due to variations in chemical, rheological, and sensory attributes. Besides, addition in the level of dietary fiber, legumes also result an increase in micronutrients. Moreover, there were several factors such as preparation of dough, protein content & quality, diastatic activity, water absorption and damaged starch, which influence the quality of product [13]. In another study barley flour mixed with wheat flour produced a better overall bread quality that was acceptable to the consumers [46].

Physical Parameters: The rats fed on guar gum had reduced feed intake, thus concluding that the guar gum could potentially be effective against obesity in humans [9]. In addition, guar gum and oat beta-glucan reduced the feed intake in Sprague Dawley rats [47]. Some other researcher's work also favored the existing results, as ingestion of palatable granulated guar gum (10 g twice a day) in obese subjects, reduced hunger significantly better than commercially available bran taken in the same way [21]. The long-term effects of guar gum in male adolescent rats were also observed that guar gum group consumed less diet throughout the entire study [28]. Lately it was also proved that guar gum induced a significant decrease in feed intake [48].

Guar gum fed rats had reduced gain in body weight since guar gum is a rich source of soluble dietary fiber [9] and present findings proved that addition of guar gum in the diet is supportive to diminish the gain in body weight, which is ultimately helpful to control overall

body weight. It has already been established from the various studies that dietary fiber may have some potential in the management of weight loss. This effect is derived from the potential influence of fiber on several aspects of food intake and nutrient availability [22]. The effects on weight loss are often deduced from effects on satiety, decreased caloric intake, and increased fecal excretion of energy in the form of fat and nitrogen [23-25]. Gel forming fibers such as guar gum is more effective in promoting weight reduction than non-gel forming fibers like wheat bran [26]. The guar gum group consumed less diet throughout the study period and gained less weight over the first 20 weeks compared to the cellulose and bran groups [28] just like the present efforts.

Research trials conducted, explored that body weight was significantly reduced during guar gum treatment (10g twice daily) [21]. Later on modified guar gum (GG) appeared to affective in appetite and body weight (BW) loss in humans [30]. Semi solid meal with GG reduced the body weight i.e. 5.6 ± 1.0 kg. Daily intake of guar gum results in a permanent weight loss. In contrast, a gain in body weight of rats fed on fortified samples of wheat with gram flour [31]. Weight gain over the balanced period and food conversion ratio decreased, linearly with increasing guar gum intake [32]. Fiber consisted of guar gum, gum Arabic, locust bean gum, pectin, and oat fiber reduced body weight by 6 pounds [49]. Moreover gain in body weight of animals fed on diets with pulse starches (red gram, black gram, green gram, Bengal gram) was observed and found to be significantly lower as compared to body weight gain with cereal starch (maize, wheat, rice, jowar, ragi) diet [50]. Recently a significant decrease in weight gain was also concluded by guar gum [48].

Organs Weight: Dietary fiber especially soluble fiber, because of their viscous and gel forming nature, showed significant affect on intestine length and weight. In this study organ, weights of different groups of rats fed on various diets prepared from composite flours resulted in greater small intestine weight relative to body weight [33]. The present conclusions are further supported by another study, which explored that diets containing 0, 10 and 20 % (w/w) guar gum (75% soluble fiber, 7.6% insoluble fibers) fed to normal male Wistar rats (107.0 ± 9.3 g) for 60 days showed 10 % increase in small intestine length and 25% retardation in the intestinal transit time [9].

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Serum Glucose: The results in present investigation are in corroboration with the previous findings of various workers, that legume and pulses are helpful in the management of serum glucose. In a study, 24.56% reduction in serum glucose, by incorporating guar in bread was reported [51]. The effectiveness of guar gum is authenticated by the fact that the hyperglycemia and hyperlipidemia can be controlled by a herbal powder consisting of guar gum, methi, tundika, and meshasringi. The herbal powder was given twice a day before major meals for four weeks [52]. The potential positive effects of chickpea in diabetes therapy and its role as biological active food supplements have been proved [53]. Metabolic response to short and long-term guar gum consumption were studied in adolescent and adult rats. Reduced plasma glucose was measured for only the guar gum group [28]. Hypoglycemic effect of guar gum indicated by the

addition of guar to an intragastric glucose load (1 g/kg) which markedly delayed the rise in plasma glucose levels when the concentration of the gum was adequate (10 mg/ml). Guar gum can reduce fasting blood glucose from 11.4 ± 3.7 mmol L^{-1} to 9.5 ± 3.9 mmol L^{-1} in rats [54, 55].

Various other researchers also proved the phenomena of hypoglycemia due to guar and guar by-products (GBP) which affected carbohydrate tolerance in rats. Both 1% and 10% GBP suspensions administered immediately before a glucose challenge (1 g/kg body weight) caused a 31% reduction in the integrated plasma glucose response area during a 180 minutes test [56]. Guar gum showed greater viscosity than the other gums during acidification and/or alkalinization and also showed larger effects on plasma glucose levels (35% reduction in maximum rise in plasma glucose) and on the total area under the curve of plasma glucose [57].

The acute effect of a single dose of guar gum has been verified to reduce the peak postprandial whole blood glucose levels (about 10%). Following long-term treatment, a further reduction was seen in the obese subjects with the highest postprandial glucose levels [21]. Clinical impact of fiber supplementation for the reduction of postprandial blood glucose showed 17% decrease in mean daily plasma glucose concentrations while the area under the curve for 2-hour plasma glucose concentrations reduced by 36 percent [49].

Serum Albumin: Serum protein values found in different groups of rats in the present investigation are in close association with hematological clinical chemistry values for male Sprague Dawley rats for total protein that ranged from 6.3-8.2 g/dL [58].Another study also reported 6.28 g/dL total protein in normal control rats [59].

Competing interests: The authors declare that there are no competing interests.

Author's Contributions: All the authors contributed equally.

CONCLUSION

Diet based strategy is a right approach as it is economical and assessable to avoid the health risks. The present research explored that diet diversification is an effective tool for the management of serum glucose and body weight. Role of legumes is indispensable to enhance the dietary fiber. Ingestion of chapaties prepared from selected compositions of composite flours providing an additional dietary fiber would be supportive to reduce hyperglycemia and obesity.

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