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# Multifunctional Ingredient Dietary Supplement for Management of Hyperglycemic and Hypercholesterolemic Therapy of Diabetes

**Pawar K**[1*] **and Thompkinson DK**[2]

[1]*Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India*

[2]*Warner School of Food and Dairy Technology, Sam Higginbottom Institute of Agriculture, Technology & Sciences, Allahabad, Uttar Pradesh, India*

[*]**Corresponding author:** Pawar K, Scientist, Department of Processing and Food Engineering, College of Agricultural Engineering and Technology, Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India, Tel: 18001803001; E-mail: kanikapawar@gmail.com

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## Abstract

A dietary supplement with multifunctional ingredients was designed, formulated and optimized using Response Surface Methodology. To evaluate the hypoglycemic and hypocholesterolemic efficacy of dietary supplement an in-vivo studies were conducted for 8 weeks. A total of thirty rats was taken and allocated to five groups with 6 rats each, First group (Group I) was given 100 percent control diet, second group (Group II) was fed with 20 percent commercial diabetic supplements while the other three experimental groups (III, IV and V) were given formulated dietary supplement substituting with control diet at 10, 20 and 50 percent. In all groups diabetes was induced by intraperitoneally administration of streptozotocin. Body weight, Blood Glucose and Cholesterol were estimated during the experimental period. Among the various dietary groups, 50 percent dietary supplementation resulted in a reduction of 16.81 percent blood glucose, 33.94 percent blood plasma cholesterol and 4.16 percent increase in body weight.

**Keywords:** Diabetes; Dietary supplement; Hyperglycemic; Hypercholesterolemic; Multifunctional; Formulation

## Introduction

Diabetes is a metabolic cum vascular syndrome of multiple etiologies characterize by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both. This disorder is frequently associated with long-term damage, which can lead to failure of organs like eyes, kidneys, nerves, heart, and blood vessels [1]. It is one of the most common metabolic disorders in the world and the prevalence of diabetes in adults has been increasing in the last decades [2]. Rapid urbanization and dramatic changes in lifestyle has driven diabetes epidemic in developing countries. As per International Diabetes Federation (IDF), globally 382 million people were diabetic in 2013 and this number is expected to rise to 592 million by 2035 whereas India was home to 65.1 million diabetics and the number will go up to a whopping 109 million by 2035 [3]. Thus there is need for health promoting policies and alternatives to encourage action to prevent diabetes in future generations.

In the midst of innovation in food and nutrition sciences, identifying and correcting nutritional deficiencies to designing foods that promote optimal health and reduce the risk of disease is on priority. The medical nutrition therapy is one such approach in managing existing diabetes, and preventing, or at least slowing, the rate of development of diabetes complications [4]. It is, therefore, important at all levels of diabetes prevention. With the advancement in existing treatments of synthetic drug therapy there is a greater risk in side effects and thus a major concern, led to alternative sources which would be safe and effective [5].

An effective way to minimize these health risks could be through beneficial nutritional interventions aimed for management of diabetes especially type 2. One of the primary goals of medical nutrition therapy is to design special dietary products for patients with hyperglycemia, intended in controlling postprandial plasma glucose fluctuations and hypercholesterolemic tendencies [4]. Dietary interventions suggested the use of constituent oral nutritional supplements sources specifically for the diabetes to improve glycemic control [6]. Currently the dietary formulations designed using a variety of substances have unusual ingredients, are therefore unable to impart the beneficial (insulinotropic, hypocholesterolemic) effect on diabetic subjects.

It is presumed that multiple functional ingredient approach could be an effective way to minimize health risks as well as their concerted role could be beneficial for the health management of individual suffering from type 2 diabetes [7]. Therefore an attempt was made, to evaluate the efficacy of formulated dietary supplement with multifunctional ingredients in management of blood glucose levels and to impart hypocholesterolemic effect through *in-vivo* studies.

## Materials and Method

### Materials

The formulated dietary supplement used in animal diet includes the Fibersym® RW resistant wheat starch which was procured from MGP, Ingredients Inc. Kansas (USA) while the Maltodextrin (DE-22-25) was supplied from M/s Rai Agro Industries Ltd., Sangrur (Punjab). Whey protein concentrate (WPC-70), casein and skim milk powder were purchased from Modern Dairy Private Ltd., Karnal (Haryana). Sodium caseinate was purchased from Avani Food Products, Mehsana (Gujarat). Splenda® Sucralose as a non-caloric sweetener was procured

from Tate and Lyle, Mumbai. Partially hydrolyzed guar gum was used as the source of soluble dietary fiber under the brand name Sunfiber® was purchased from Taiyo Lucid Pvt. Ltd., Mumbai. Ground nut oil (Amrit Banaspati Co. Ltd, Rajpura), Corn Starch and Sucrose were purchased from local markets. Vitamin and Mineral mix used in preparation of animal diet was procured from the Sd-fine Chemicals Private Limited, Mumbai.

## Methodology

**Multifunctional ingredient formulation:** The dietary supplement was formulated as described below.

**Designing and Optimization of the dietary supplement:** The basis for designing a suitable diabetic dietary supplement was undertaken in terms of total calorific requirements and the percentage of calories supplied by various ingredients as per the recommendations of ICMR (2005) for an adult diabetic patient. This recommendation suggests a range of 1500-2100 kcal diet to meet the nutritional requirement of a diabetic adult. Further, it is suggested that the fat components must supply 20-25 percent of total calories of which less than 7 percent should come from saturated fats while rest should be in the form of MUFA and PUFA in equal ratio.

The protein component should supply 10-15 percent of total calories and 55-65 percent must be compensated with carbohydrate component of the diet. Keeping in view the recommendation of ICMR a supplement diet was designed to supply basic average requirement of 1800 kcal for the diabetic subjects. The energy components were met by supplying 15, 17 and 68 percent by weight of fat, protein and carbohydrate respectively. Hence, the percent energy contribution supplied by all the three ingredients was in close proximity to the range mentioned by the ICMR for the diabetic patients.

**Selection of the ingredients:** As per nutritional requirement of fat for a diabetic adult, the source of fat was supplied through a mixture of milk fat and groundnut oil (GNO). The appropriate mixture was selected on the basis of Fatty acid composition and to provide maximum medium chain unsaturated fatty acids (MUFA) and a suitable ratio of MUFA: PUFA for a beneficial effect. Protein was provided by the whey protein concentrate -70 and sodium caseinate, so as to add all essential amino acids required for body maintenance in a diabetic adult.

Resistant wheat starch (RWS) and Maltodextrin were used in proportionate amount as source of carbohydrate. Suitable combination was selected in order to keep the concentration of resistant starch maximum limit in the supplement as it helps to control blood glucose level through slow release. The insulinotropic and hypocholesterolemic effects of the various selected ingredients used for the preparation of the dietary supplement are well reviewed by Pawar and Thompkinson, [7]. In order to arrive at an optimized formulation, different combinations of the selected ingredients as a source of fat, protein and carbohydrate content of the formulation were studied using Central composite rotatable design technique through Response Surface Methodology [8].

**Product Formulation:** The spray dried product was prepared by admixing predetermined amounts of milk fat and groundnut oil as a source of fat along with maltodextrin as source of carbohydrate; whey protein concentrate and sodium caseinate as a source of high quality protein (with functionality of fat encapsulators) along with partially hydrolysed guar gum (PHGG), as a source of soluble dietary fibre with a calculated amount of skim milk. The contents were mixed well at 60-70°C. The mixture was then homogenized at 2000 and 500 psi in a double stage homogenizer followed by spray drying at an inlet temperature of 200°C and outlet temperature of 90°C. The spray-dried product was cooled to room temperature and calculated amount of resistant wheat starch; sweetener (sucralose) and cocoa powder as flavoring were dry blended to the spray dried product followed by packaging and storage.

**Animal (*In-vivo*) Studies:** To evaluate the efficacy of the preparation multifunctional ingredient formulation *in-vivo* studies were carried out on experimental animals as described below. The formulated diabetic dietary supplement was fed to the diabetes induced experimental animals and in vivo studies were done using standard protocol.

**Feeding Protocol:** The dietary supplement was fed to animal models for a period of 8 weeks to study its hyperglycemic properties. Five groups of male albino rats were taken and comprising of 6 rats in each group. The control group I was given the synthetic (control) diet of the composition as mentioned in Table 1. The control group II was fed with the 20 percent commercial or market diabetic supplement to study it's hypoglycemic and hypocholesterolemic effect and to compare it with the prepared dietary formulation. The other three experimental groups were given the formulated diabetic supplement substituting with control diet at 10, 20 and 50 percent level as represented in Figure 1. The composition of the formulated dietary supplement is given in Table 2. The composition of the vitamin and mineral mixture [9] used in the preparation of the synthetic (control) diet are given in Tables 3a and 3b.

**Animal maintenance and experimental design:** The *in-vivo* experiments were conducted in the Deshpande Laboratories Private Limited., Bhopal (Madhya Pradesh). In addition to the experimental protocol was approved from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi. Thirty male albino rats with an average weight of 140+5 g were housed in a stainless steel cage placed in a room temperature maintained at 24+2°C with a 12 h light/12 h dark cycle and 60+5 percent relative humidity.

All animals were fed on a standard diet for two weeks. After this acclimatization period, the rats were divided randomly into five experimental groups consisting of six rats each. In all the groups diabetes was induced by administration of streptozotocin (Sigma Chemical Co., Louis, MO, USA) dissolved in 0.01 M sodium citrate buffer (pH 4.5) at a dose of 150 mg/kg intraperitonial injection in sterile saline. After 7 days of administering streptozotocin, diabetes was confirmed in the groups by checking the blood glucose levels using Accu-Chek active blood glucose meter (Roche Diagnostic India Pvt. Ltd.) [10].

**Assessment of body weight:** Body weight of animals from all the group animals was measured at a weekly interval.

The blood glucose levels of all groups of animals were determined at weekly intervals during the experimental period on 12 hrs fasted rats. Blood samples were drawn from the tail by pricking it using a Lancet. The blood glucose level was measured by Accu Check active blood glucose meter (Roche Diagnostic India Pvt. Ltd.).

**Blood collection and cholesterol estimation:** At the beginning and end of the experiment, blood samples were collected in heparinized tubes (200 U/μL) from the eyes from 12 hrs fasted rats and centrifuged at 4000 rpm for 10 min. The plasma obtained was used to analyze the

cholesterol in the blood samples. The cholesterol was analyzed using cholesterol estimation kit.

**Statistical analysis:** All data obtained was analyzed using the software application programmers (Microsoft Excel 2007). The results of the studies were expressed as mean ± standard error. Significance was tested by employing analysis of variance (ANOVA) and comparison between means was made by critical difference (C.D) value.

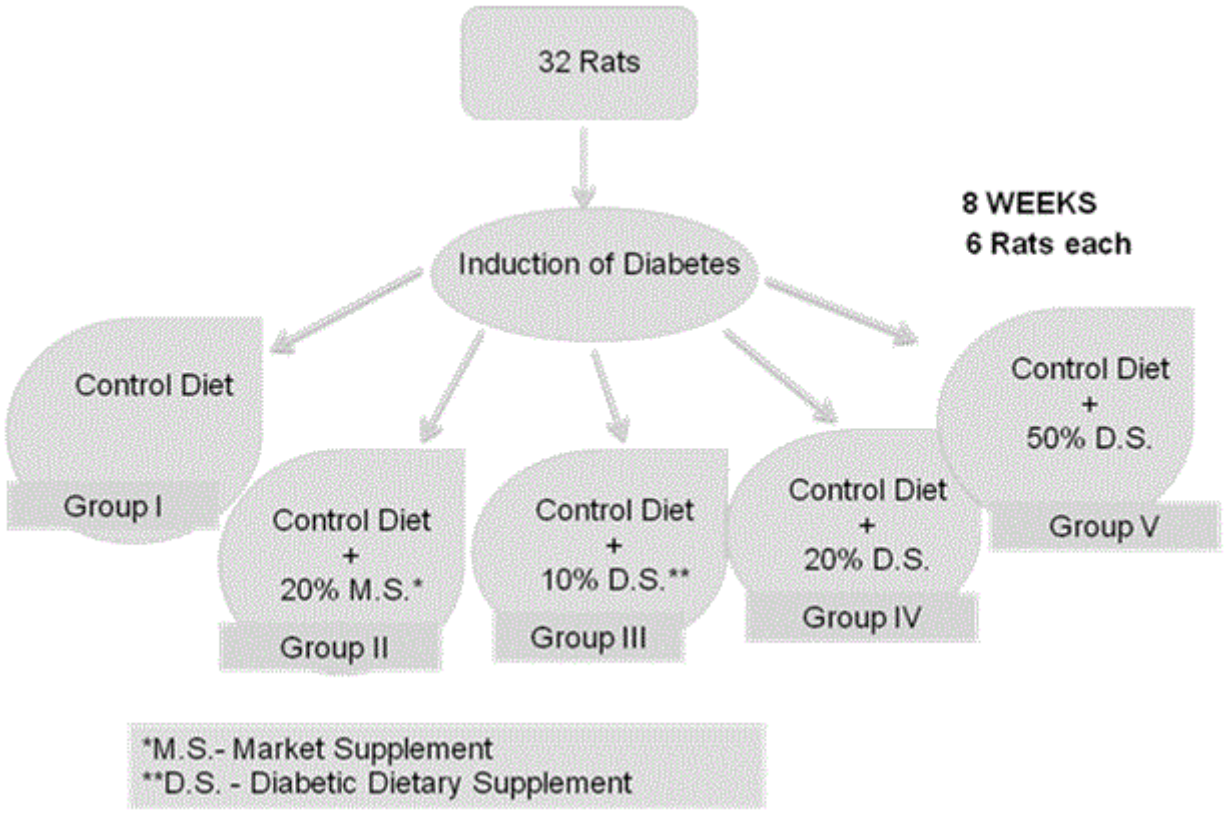


**Figure 1:** Schematic representations of the groups along with the feeding proportions.

| Ingredients | Percent |
|---|---|
| Starch | 58 |
| Casein | 18.5 |
| Sucrose | 7.5 |
| Groundnut oil | 8.5 |
| Cellulose fiber | 4.5 |
| Mineral mixture | 3.7 |
| Vitamin mixture | 1.1 |
| Choline chloride | 0.01 |
| Methionine | 0.3 |

**Table 1:** Composition of the synthetic (control) diet (per cent of diet).

| Ingredients | 100 kg |
|---|---|
| Groundnut oil | 9 |
| Milk fat | 1 |
| Resistant wheat starch | 37.7 |
| Maltodextrin | 18.6 |
| Skim milk powder | 11.34 |
| Whey protein conc.-70 | 11.34 |
| Sodium caseinate | 4.32 |
| Partially hydrolysed guar gum | 7 |
| Cocoa powder | 4 |
| Sweetener | 0.02 |

**Table 2:** Composition of diabetic dietary supplement.

| Vitamin mixture | mg / 100 g |
|---|---|
| Vitamin A | 2000 IU |
| Vitamin D | 200 IU |
| Vitamin K | 0.500 mg |
| Vitamin E | 100 IU |

| | |
|---|---|
| PABA | 10.00 mg |
| Mesoinositol | 10.00 mg |
| Niacin | 4.00 mg |
| Ca-D-Pantothenate | 4.00 mg |
| Riboflavin | 0.800mg |
| Thiamin-HCl | 0.500 mg |
| Pyridoxin-HCl | 0.500 mg |
| Folic acid | 0.200 mg |
| Biotin | 0.040 mg |
| Vitamin B12 | 0.003 mg |

**Table 3a:** Composition of Vitamin Mixture (100 g) AOAC [9].

| Salt mixture | g/kg |
|---|---|
| $KH_2PO_4$ | 389.00 g |
| $MgSO_4$ | 57.30 g |
| $CaCO_3$ | 381.40 g |
| $FeSO_4.7H_2O$ | 27.00 g |
| $MnSO_4.H_2O$ | 4.100 g |
| $ZnSO_4.7H_2O$ | 0.548 g |
| $CuSO_4.5H_2O$ | 0.477 g |
| $CoCl_2.6H_2O$ | 0.023 g |
| NaCl | 139.30 g |
| KI | 0.79 g |

**Table 3b:** Composition of Mineral Mixture (1 kg) AOAC [9].

## Results and Discussion

### Effect on body weight

The effect of feeding diabetic multifunctional ingredient dietary supplement on the body weight of experimental animals was determined through feeding regime of 35 days. The body weight of experimental animals was monitored at weekly intervals. The data related to the effect of feeding different levels of diabetic dietary supplement on the body weight of different groups of experimental animals are presented in Table 4.

| Groups | Days | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 | 35 | $C.D_{0.05}$ |
| Group I | $143.56^{an} \pm 1.54$ | $143.11^{ao} \pm 1.52$ | $145.08^{bo} \pm 1.76$ | $145.44^{bco} \pm 1.66$ | $146.69^{cdo} \pm 1.84$ | $147.59^{do} \pm 2.09$ | 1.45 |
| Group II | $143.92^{an} \pm 1.32$ | $144.63^{abno} \pm 1.11$ | $145.98^{bno} \pm 1.15$ | $148.22^{c} \pm 1.22$ | $149.56^{cdno} \pm 0.79$ | $150.37^{dno} \pm 1.12$ | 1.48 |
| Group III | $146.07^{an} \pm 0.53$ | $147.23^{bn} \pm 0.55$ | $149.21^{cmn} \pm 0.70$ | $150.73^{d} \pm 0.63$ | $152.88^{emn} \pm 0.64$ | $153.15^{em} \pm 0.62$ | 1.14 |
| Group IV | $151.72^{am} \pm 0.75$ | $151.45^{am} \pm 0.85$ | $152.25^{am} \pm 0.79$ | $154.23^{b} \pm 0.74$ | $155.12^{bm} \pm 1.06$ | $154.68^{bm} \pm 1.13$ | 1.35 |
| Group V | $146.69^{an} \pm 1.65$ | $147.50^{abn} \pm 1.93$ | $148.58^{bcmno} \pm 1.97$ | $149.83^{cd} \pm 1.49$ | $151.36^{den} \pm 1.43$ | $152.79^{emn} \pm 1.67$ | 1.78 |
| $C.D_{0.05}$ | 3.46 | 3.85 | 4.11 | 3.49 | 3.59 | 3.9 | |

**Table 4:** Average Body weight (g) of the animals with different diets at selected time periods. Mean ± S.E. Figures are average values of six rats per group. $^{abcde}$Mean ± SE with different superscripts within columns differ significantly ($p \leq 0.05$). Group I: Control diet, Group II: 80% Control Diet +20% Market Supplement, Group III: 90% Control Diet+10% Diabetic Dietary Supplement, Group IV: 80% Control Diet+20% Diabetic Dietary Supplement, Group V: 50% Control Diet+50% Diabetic Dietary Supplement.

All the five groups of animals were induced with the diabetes and after 7 days of the induction, the initial body weight was monitored. When the experimental animals were found to afflict with the diabetes disease, it was considered the starting day of the experiment.

At the 0th day, average body weight of the diabetic experimental animals ranged from 143.56 to 151.72 g. The maximum body weight of 151.72 g was observed in the group IV with 20 percent supplementation, followed by body weight of 146.69 and 146.07 g in group V receiving 50 percent supplementation and group III with 10 percent respectively.

The group II with the 20 percent commercial dietary supplement found to have a body weight of 143.92 g whereas control group I showed minimum weight of 143.56. After 7 days of feeding the average body weight were found to be maximum (151.45 g) in the Group IV followed by groups III (147.23 g) and group V (147.50 g). While a minimum weight (143.11) was recorded in the control group. The average body weight values on 14th day ranged from 145.08 to 152.25 g.

The group IV (152.25 g) was found to have maximum body weight followed by group III (149.21 g) and group V (148.58 g). While the group II and group I was found to comprise of minimum body weight of 145.98 g and 145.08 g respectively. Average body weight monitored on the 21st day, 28th day and 35th day revealed that the body weight varied from 145.44 to 154.23 g, 146.69 to 155.12 g and 147.59 to 154.68 g respectively within the groups. The maximum body weight was (155.12 g) found in group IV followed by group III (154.23 g) and Group V (147.59 g). While minimum weight (145.44 g) was recorded in the group II and group I after 35 days of feeding.

Statistically it was found that, on the 7th day of feeding the experimental diet, there was a non-significant increase in body weight between group I, II, IV and V.

However a significant ($p \leq 0.05$) increase in the body weight was noticed in the group III. Between 7th and 14th day a non-significant change was observed in group II, group IV and group V while a significant ($p \leq 0.05$) increase was observed in the group III and I. Between 14th and 21st day, a significant increase in body weight was found in the groups II, III and IV while group I and V was found to have non-significant increase.

On the 21st day, a significant increase was observed for group III while a non-significant change was found in group IV. However, by the end of the feeding regime of 35 days a significant ($p \leq 0.05$) increase was observed from the initial body weight in all the dietary groups.

Among all the experimental groups, the group III with 10 percent dietary supplementation showed significant ($p \leq 0.05$) increase in the body weight during 35 days of feeding regime. While a minimum increase in the body weight was found in the group IV with 20 percent supplementation which showed significant increase between 14th to 21st days.

Maximum increase in the body weight was observed in group III which may be due to the least degree of supplementation (10 percent) while as the percent dietary supplement contribution was increased a lower increase in body weight may have been due to more fibre in the diet as it was observed in the group IV and group V.

Further, the statistical analysis as indicated in Table 4 shows that the increase in all dietary groups was significant ($p \leq 0.05$) after 35 days of feeding regime. As the resistant wheat starch is considered as non-digestible portion of the diet and hence contributing lesser calories resulted in the small increase in body weight reported among the group V and IV. Further the percent increase in body weight among different experimental groups during feeding period of 35 days is depicted in Figure 2.

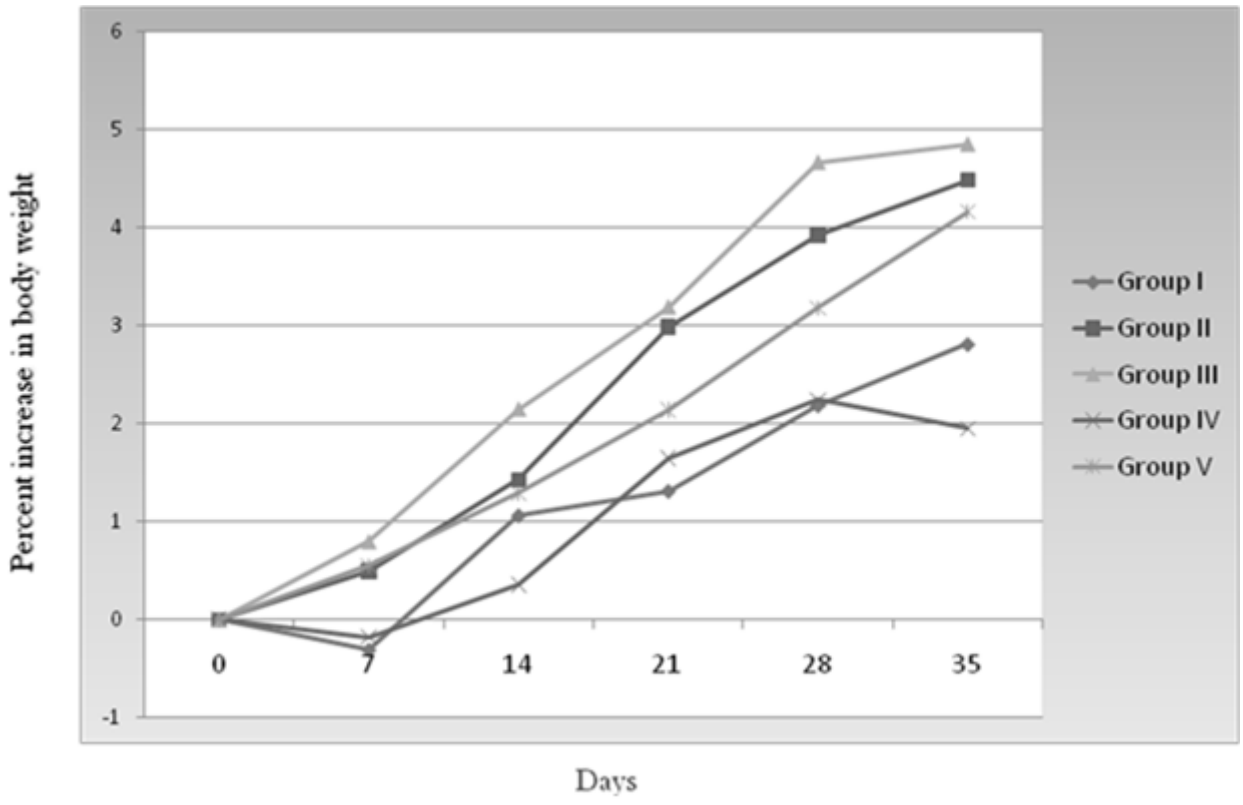


**Figure 2:** Increase in body weight among the different experimental groups at selected time period.

The maximum increase of 4.85 percent was found in group III where the body weight increased from 146.07 to 153.15 g. This was followed by group II with 4.49 percent and group V with 4.16 percent increase in body weight. While the minimum percent increase in body weight was observed in group I and group IV with 2.81 and 1.95 respectively. This could be due to the lesser proportion of the major ingredients like carbohydrates, protein and fat and other several ingredients which may possibly affect the body weight and contribute to the overall diet of the healthy animal.

The body weight of animals decreased as the percent supplementation was increased. This could be due to the higher percent contributed by resistant starch which helps burn fat and may lead to lower fat accumulation. A recent clinical trial with high amylose corn resistant starch showed that it increased fat oxidation after a meal [11]. It also changed the sequence in which the body burns food with fat burning being placed at the top of the list relative to **carbohydrates** and **protein**. These findings suggest a possible **metabolic** effect of resistant starch that may impact body weight [11].

Koo et al. [12] also found that the addition of the chemically modified resistant starch namely Cross Linked Corn starch-5 (CLCS) and Cross Linked Corn Starch-12 to the high-fat diet (HFD) had significant effects on the mice final body weight which was significantly reduced compared to the HFD group. In addition, a 64.10 percent and 54.18 percent reduction in the body weight was observed in the CLCS-5 and -12 groups, respectively, compared with the HFD group at the end of the trial. This result was favorably compared with the study of Jeong et al. [13] where the diets containing resistant starch caused a reduced weight gain by 7 percent.

It would be explained by the fact that resistant starch decrease energy absorption, thus giving rise to a decrease in epididymal fat pads and serum triacylglycerol concentration [14]. Previous studies of Cheng and Lai [15]; Ebihara et al. [16]; Ranhotra et al. [17] showed that the diet containing resistant starch reduced the organ weight such as liver. Hence, these results would be possibly from the inclusion of resistant starch in the diets.

The decrease in body weight in diabetic rats shows that the loss or degradation of structural proteins is due to diabetes, and the structural proteins are known to contribute to the body weight [18]. Eventually, from the results it can be concluded that group III has reported a higher percent increase in the body weight as compared to all the dietary experimental groups due to lesser percent (10%) supplementation of the formulation prepared which is in proportion to the lower percent of resistant starch in their diets.

## Effect on blood glucose levels

Initially, at the time of induction of the diabetes in the experimental animals the blood glucose levels of all the groups were analyzed. The blood glucose levels in the normal animals ranged from 70.17 to 73.50 mg/dl. To execute the hypoglycaemic effects of feeding diabetic dietary supplement, the blood glucose levels of the experimental animals was monitored at weekly intervals throughout the feeding regime. The data related to the effect of feeding different levels of diabetic dietary supplement on the blood glucose levels of the experimental animals is presented in Table 5.

| Groups | Days | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | -7 | 0 | 7 | 14 | 21 | 28 | 35 | $C.D_{0.05}$ |
| Group I | $71.67^{am} \pm 0.56$ | $151.50^{bm} \pm 2.78$ | $166.00^{cm} \pm 3.75$ | $168.00^{cm} \pm 3.36$ | $173.17^{cdm} \pm 2.41$ | $175.83^{dm} \pm 2.93$ | $172.83^{dm} \pm 2.43$ | 7.2 |
| Group II | $73.50^{am} \pm 1.12$ | $155.83^{bm} \pm 1.14$ | $154.33^{bn} \pm 1.43$ | $144.17^{cp} \pm 4.26$ | $144.67^{co} \pm 2.55$ | $138.83^{cn} \pm 5.68$ | $141.17^{cn} \pm 4.64$ | 9.5 |
| Group III | $70.17^{an} \pm 0.48$ | $155.17^{bm} \pm 0.54$ | $158.17^{bcn} \pm 1.42$ | $159.17^{cn} \pm 1.14$ | $169.67^{dm} \pm 1.69$ | $178.67^{em} \pm 0.99$ | $176.17^{em} \pm 1.49$ | 3 |
| Group IV | $73.17^{am} \pm 0.48$ | $154.17^{bm} \pm 1.49$ | $153.67^{bn} \pm 0.84$ | $153.83^{bno} \pm 2.09$ | $152.67^{bcn} \pm 2.75$ | $148.17^{cn} \pm 2.01$ | $149.67^{cn} \pm 1.98$ | 5.1 |
| Group V | $72.00^{am} \pm 1.13$ | $155.67^{bm} \pm 1.48$ | $153.17^{bn} \pm 1.99$ | $148.50^{bop} \pm 3.41$ | $130.83^{cp} \pm 2.50$ | $123.00^{do} \pm 1.93$ | $129.50^{do} \pm 2.96$ | 7.2 |
| $C.D_{0.05}$ | 2.13 | 5.14 | 6.82 | 8.27 | 7.21 | 10.09 | 9.03 | |

**Table 5:** Average Blood glucose levels (mg/dl) with different diets at selected time periods. Mean ± S.E. Figures are average values of six rats per group. $^{abcde}$Mean ± SE with different superscripts within columns differ significantly ($p \leq 0.05$). $^{mnopq}$Mean ± SE with different superscripts within rows differ significantly ($p \leq 0.05$). Group I: Control diet. Group II: 80% Control Diet+20% Market Supplement. Group III: 90% Control Diet+10% Diabetic Dietary Supplement. Group IV: 80% Control Diet+20% Diabetic Dietary Supplement. Group V: 50% Control Diet+50% Diabetic Dietary Supplement.

After 7 days of the induction period the blood glucose levels of all five groups of experimental animals were analyzed. When the animals were found to be diabetic (blood glucose level >126 mg/dl) it was considered the starting day (i.e., zero day) of the experiments. At the zero days the animals were found to have the higher blood glucose levels ranging from 151.50 to 155.83 mg/dl. As the blood glucose levels was observed to be >126 mg/dl, thus the experimental animals were considered diabetic. Now the effect of different levels of diabetic dietary supplement along with the level of commercial dietary supplement on the blood glucose levels of the diabetic subjects was monitored. The results are given in Table 5.

At the 7th day, the blood glucose level of the diabetic experimental animals ranged from 153.17 to 166.00 mg/dl. The high blood glucose level of 166.00 mg/dl was observed in the control group I, followed by the group III with 10 percent supplementation (158.17 mg/dl). And group II with 20 percent commercial supplementation (154.33 mg/dl). The blood glucose level of 153.67 and 153.17 mg/dl was found for group IV and V which was lowest among all the groups. The control group was found to have the higher blood glucose level (168.00 to 172.8 mg/dl) throughout the feeding regime which was followed by group III (159.17 mg/dl) and group IV (153.83 mg/dl).

Lowest blood glucose levels were found in group V (148.50 mg/dl) and group II (144.17 mg/dl) which were comparable to each other. Further along the subsequent days it was observed that the highest blood glucose level was in control group (173.17, 175.83 and 172.83 mg/dl) followed by the group III (169.67, 178.67 and 176.17 mg/dl); group IV (152.67, 148.17, and 149.67 mg/dl) and group II (144.67, 138.83 and 141.17 mg/dl) while a lowest blood glucose level (130.83, 123.00 and 129.50 mg/dl) was observed for group V on the 21st, 28th and 35th day respectively. Among all the groups II, IV and V have reported a decreasing trend of blood glucose levels as compared to group I and group III. The blood glucose levels decreased from 155.83 to 141.17 mg/dl in group II; 154.17 to 149.67 mg/dl in group IV and 155.67 to 129.50 mg/dl in the group V while the blood glucose levels increased in case of group I and group III from 166.00 to 172.83 mg/dl and 158.17 to 176.17 mg/dl respectively.

The data presented in Table 5 further indicates that during the feeding regime of 35 days, there has been observed a significant ($p \leq$

0.05) increase in the blood glucose level in control group I and group III while a significant decrease ($p \leq 0.05$) in the blood glucose levels were found in the group II, group IV and group V. Statistically it can also be seen from table 5 that, there was a significant increase in the blood glucose level from 0th to 7th day in the group I.

Thereafter a non-significant increase in blood glucose level was observed from 14th to 35th day. For group II, between 0th and 7th day a non-significant decrease was followed by a significant decrease ($p \leq 0.05$) on the 14th day. Thereafter a non-significant decrease was observed on the subsequent 21st, 28th and 35th day. The dietary group III has shown a non-significant decrease between 0th to14th days. However between 14th and 21st day a significant ($p \leq 0.05$) decrease was observed. Similarly, for the group IV, a non-significant decrease from 0th to 21st day followed by a significant ($p \leq 0.05$) decrease was observed at the end of feeding regime. While in case of group V, a non-significant decrease from 0th to 14th day was followed by a significant ($p \leq 0.05$) decrease from 21st day till the end of the feeding period was observed.

It can also be inferred from Table 5 that the level of blood glucose in group V was significantly lower ($p \leq 0.05$) from all the groups during the study period of 35 days. In the control group I blood glucose level was significantly higher ($p \leq 0.05$) among all the groups followed by the group III. The increase in blood glucose levels in group III and group I having 13.53 and 14.04 percent respectively.

This may be due to the absence of the hypoglycemic constituents (resistant wheat starch, milk proteins and fibre) in the control group while the amount of these constituents were not sufficient in the group III with only 10 percent supplementation, hence not able to exert the beneficial effect on the diabetic subjects and resulted in the increase in the blood glucose levels. However the results of group II and group IV have shown that 20 percent supplementation of both the prepared dietary formulation and commercial dietary supplement have resulted in the significant decreasing effect on the experimental animals.

Figure 3 also depicts the percent reduction in blood glucose levels among different experimental dietary groups during feeding period of 35 days. As it can be seen from fig 3 that the maximum reduction in blood glucose of 16.81 percent was observed in group V with 50 percent dietary supplementation, followed by a 9.41 percent reduction in group II and 2.92 percent reduction in group IV.

This maximum blood glucose lowering effect observed in group V may have been due the presence of sufficient amount of resistant wheat starch along with the milk proteins which also have an insulinotropic effect and thus preventing the hyperglycemic tendencies. Both these constituents are capable of reducing the glucose level of blood plasma.

It has been reported by Granfeldt et al. [19], that the meals containing high amylose resistant cornstarch (70-75 percent amylose) have been shown to reduce postprandial glucose and insulin responses (57 and 42 percent lower, respectively) in healthy subjects as compared to meals containing ordinary cornstarch. Tamimi et al. [20] also compared the postprandial glycemic and insulinemic responses to nutrition bars containing either cross-linked RS type 4 (RS4XL) or standard wheat starch in normoglycemic adults.

The volunteers consumed a glucose beverage (GLU), a puffed wheat control bar (PWB), and a bar containing cross-linked RS4 (RS4XL) matched to available carbohydrate content. The RS4XL peak glucose and insulin concentrations were lower than the GLU and PWB. The incremental area under the curve (iAUC) for glucose and insulin were lower following ingestion of RS4 as compared with the GLU and PWB. Similarly, Nilsson et al. [21] has reported that milk proteins, in particular the whey fraction and casein [22], have a stimulating effect on insulin secretion in healthy subjects.

Frid et al. [23] evaluated that the supplementation of meals having a high glycemic index (GI) with whey proteins may increase insulin secretion and improve blood glucose control in type 2 diabetic subjects. The insulinotropic action of casein has been showed by Westphal et al. [24] which have found that the incorporation of 50 g sodium caseinate in mixed meal showed an increase in the insulin area under curve by 29 percent up to 3 hrs and thus resulted in insulin secretion. Thus due to both these constituents the formulated supplement would able to exert the decreasing effect on the blood glucose levels. From the results it can be concluded that the group V with 50 percent supplementation has reported a maximum decreasing effect on the blood glucose levels.

## Effect on blood cholesterol

To execute the hypocholesterolemic effects of feeding diabetic dietary supplement in the diet, the blood plasma cholesterol was analyzed at the beginning and at the end of the feeding regime.

The data related to the effect of feeding different levels of diabetic dietary supplement on the plasma cholesterol level of experimental animals is presented in Table 6. At the starting day of the experiments, the initial blood plasma cholesterol level of the diabetic induced experimental animals ranged from 252.30 to 265.20 mg/dl. The high initial cholesterol level was resulted due to the more tendencies of the diabetic subjects for the hypercholesterolemic abnormalities.

The maximum level of 265.20 mg/dl was present in the group V followed by almost similar levels of 262.80 mg/dl and 262.20 mg/dl in group III and the control group respectively. The cholesterol level of group II with the 20 percent commercial dietary supplement was reported a 254.20 mg/dl whereas, group IV with 20 percent dietary supplement showed minimum levels of 252.30 mg/dl.

There was no significant difference among the values of all these groups at 0 day as is evident from statistical analysis presented in Table 6. At 35th days of feeding the experimental diet, there was found a sharp decrease in cholesterol level in all the groups with the exception in control group. At the end of the experiments, the final blood plasma cholesterol level of the diabetic induced experimental animals ranged from 175.20 to 266.20 mg/dl.

Group I with control diet rather showed increase in blood cholesterol from 262.20 mg/dl to 266.00 mg/dl during 35 days of feeding regime. However, the cholesterol level of all other groups showed a decreasing trend, with maximum decrease in group V receiving experimental diet followed by Group II when received commercial diabetic diet. The group III with the 10 percent dietary supplement was observed to have 253.20 mg/dl cholesterol level whereas, group V with 50 percent dietary supplement showed minimum levels of 175.20 mg/dl.

Further, the statistical analysis as indicated in Table 6 shows that the decrease in all groups was significant ($P \leq 0.05$) after 35 days of feeding except the group I with control diet and group III with 10 percent dietary supplementation. The control group has reported a non-significant change in the cholesterol levels. This could be due to the absence of diabetic dietary supplement in the diet which due to its constituents has hypocholesterolemic effect. However, in the group II

with the 20 percent commercial supplement the cholesterol levels significantly ($p \leq 0.05$) decreased from 254.20 to 212.30 mg/dl which is comparable to the results found with the 20 percent supplementation of the prepared dietary formulation in the group IV (252.30 to 211.70 mg/dl).

A significant ($p \leq 0.05$) decrease was also observed in the group V with 50 percent dietary supplementation from 265.20 mg/dl to 175.20 mg/dl while a non-significant change was noticed in the group III with 10 percent supplementation. The experimental groups (II & IV) having 20 percent supplementation of both commercial as well as the prepared dietary formulation exhibited the similar cholesterol lowering effect. Figure 4 also depicts the change in average plasma cholesterol levels among different dietary groups at selected time periods.

The maximum reduction in blood plasma cholesterol of 33.94 percent was observed in group V with 50 percent dietary supplementation, followed by a 16.48 percent reduction in group II and 16.09 percent reduction in group IV. While the minimum reduction in cholesterol levels was noticed in group III with 10 percent supplementation, in contrast group I was resulted in 1.4 percent increase in the cholesterol levels.s

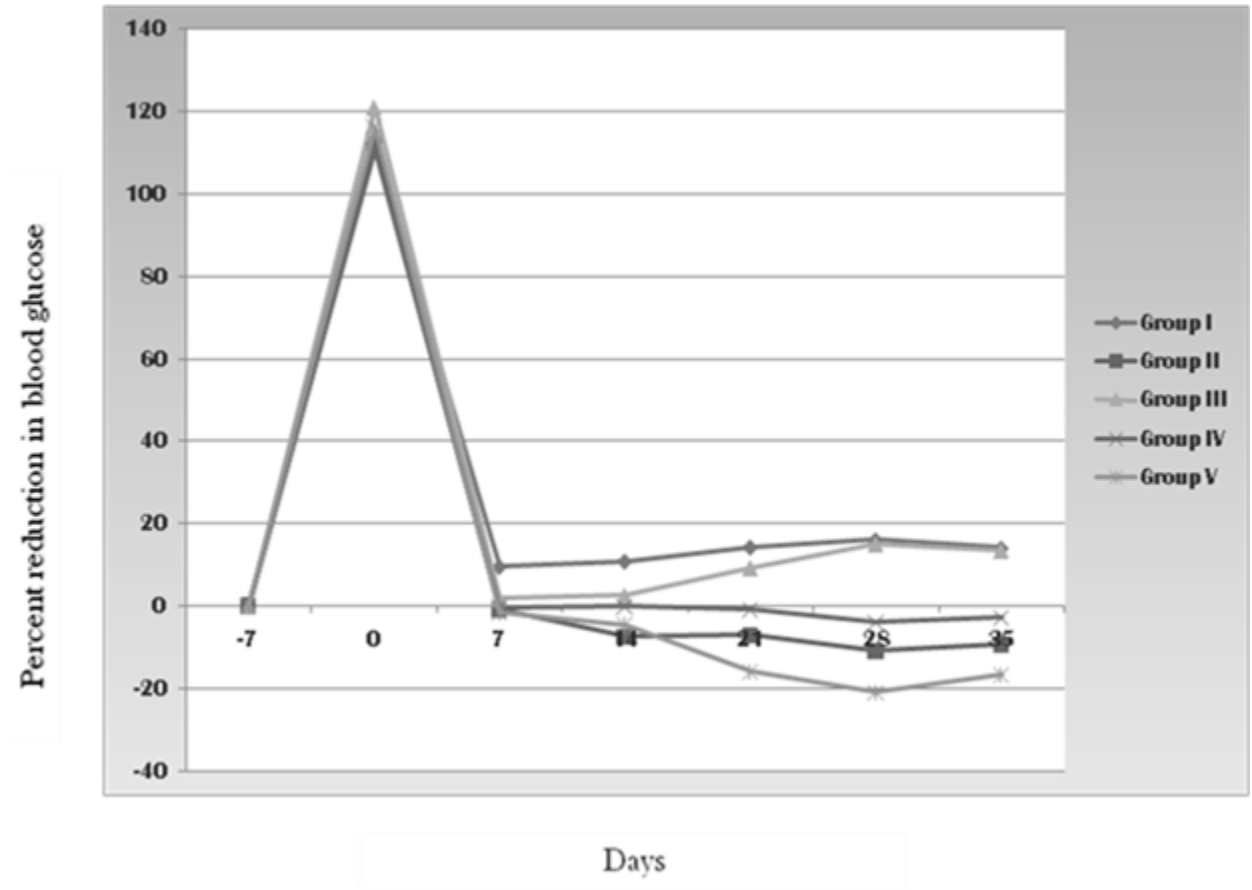


**Figure 3:** Reduction in blood glucose levels among the different experimental groups at selected time period.

| Groups | Dietary Groups | 0th day | 35th day | $C.D_{0.05}$ |
|---|---|---|---|---|
| Group I | (Control diet ) | $262.20^{am} \pm 4.90$ | $266.00^{am} \pm 2.80$ | 15.6 |
| Group II | (80% C.D.* + 20% M.S.**) | $254.20^{am} \pm 5.10$ | $212.30^{bn} \pm 6.30$ | 24 |
| Group III | (90% C.D. + 10% D.S.***) | $262.80^{am} \pm 5.20$ | $253.20^{am} \pm 4.20$ | 11.8 |
| Group IV | (80% C.D. + 20% D.S.) | $252.30^{am} \pm 6.10$ | $211.70^{bn} \pm 6.30$ | 19.6 |
| Group V | (50% C.D. + 50% D.S.) | $265.20^{am} \pm 4.20$ | $175.20^{bo} \pm 3.10$ | 16.9 |
| C.D0.05 | | 13.44 | 14.63 | |

**Table 6:** Average Cholesterol levels (mg/dl) of the animals with different diets at selected time periods. Mean ± S.E. Figures are average values of six rats per group. $^{ab}$Mean ± SE with different superscripts within columns differ significantly ($p \leq 0.05$). $^{mno}$Mean ± SE with different superscripts within rows differ significantly ($p \leq 0.05$). *C.D.-Control Diet, **M.S.-Market Supplement, ***D.S-Diabetic Dietary Supplement.

As per the recommendations of the Indian Council of Medical Research the total cholesterol level should be <180 mg/dl [1] for the diabetic subjects. The effect of 50 percent supplementation resulted in a greater cholesterol reducing effect (175.20 mg/dl) on the experimental

animals which is in line with ICMR recommendations as compared to the commercial diabetic formulation.

This high cholesterol lowering effect observed in group V, fed diet containing 50% dietary supplement, may have been due to the presence of sufficient unsaturated fatty acids and in particular monounsaturated fatty acid in the lipid portion of dietary supplement as well as the presence of sufficient amount of the dietary fibre in the form of partially hydrolysed guar gum. Both these constituents are capable of reducing the total cholesterol level of blood plasma (Figure 4).

The presence of these two cholesterol lowering ingredient may have been responsible in exhibiting hypocholesterolemic effect of the experimental diet. Vessby et al. [25] found that foods containing monounsaturated fats lower **LDL** cholesterol, while possibly raising **HDL** cholesterol. Ramesh et al. [26] also observed that the normal and diabetic rats fed on 8 percent groundnut oil diet, resulted in a significant reduction in Total Cholesterol (TC), Very Low Density Lipoprotein-Cholesterol (VLDL-C), Low Density Lipoprotein-Cholesterol (LDL-C), Triglycerides (TG) and an elevation in High Density Lipoprotein-Cholesterol (HDL-C).

Kuo et al. [27] have reported that dietary supplementation with PHGG in hamsters fed with high unsaturated fat diet reduced plasma cholesterol and lipid profiles. Other researchers found that PHGG supplementation decreased cholesterol and lipid levels, thus it has a potential to prevent hypertension and cardiovascular diseases [28-30].

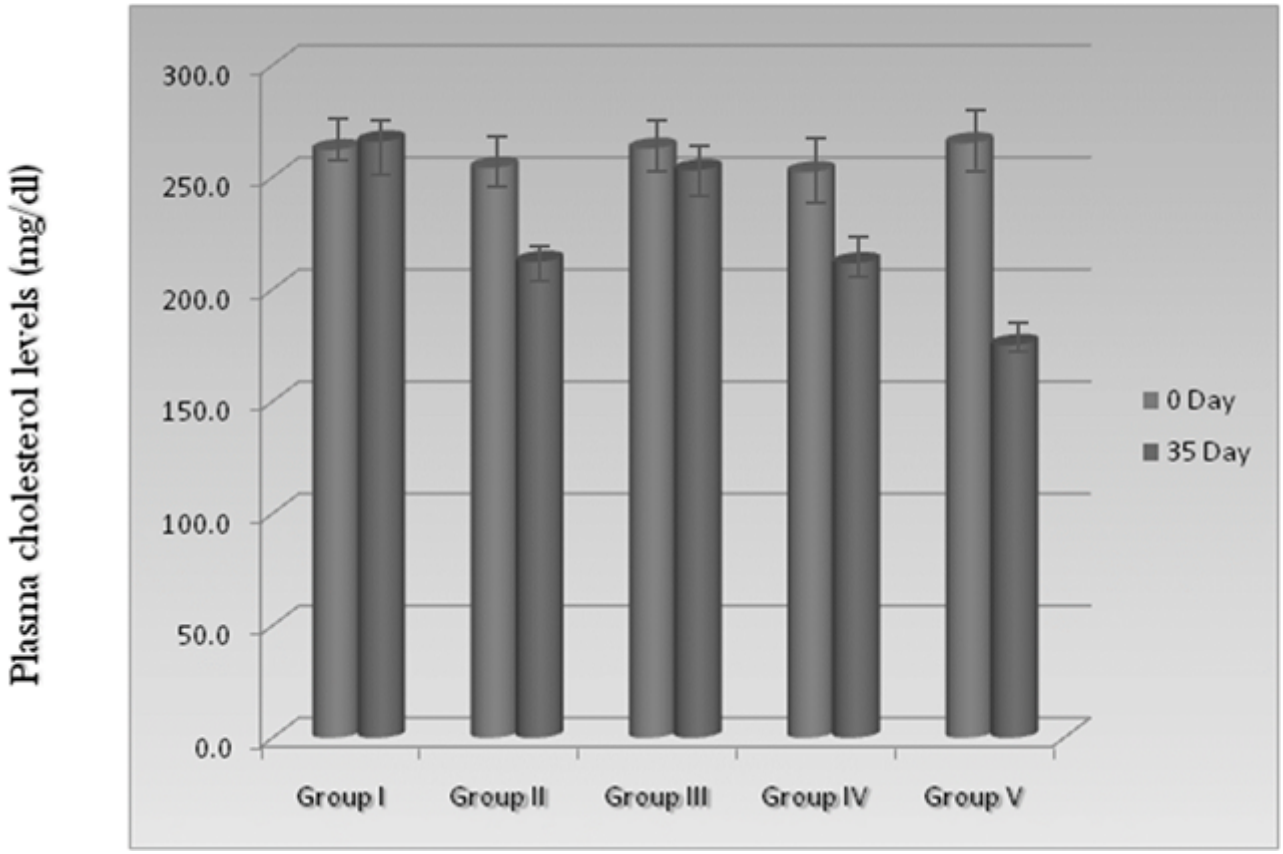


**Figure 4:** Reduction in blood plasma cholesterol levels among the different experimental groups at selected time period.

It was also observed by Sierra et al. [31] that consumption of 14 g/d of psyllium in twenty type 2 diabetic patients for 6 weeks resulted in reduction of total and LDL-cholesterol by 7 percent and 9 percent, respectively. Khan et al. [32] observed a large reduction (25 percent) in LDL-cholesterol in 24 healthy volunteers receiving 9 g of guar gum per day for 4 weeks. In addition, cocoa powder and dark chocolate also affect cardiovascular disease risk status by modestly reducing LDL oxidation susceptibility, increasing serum total antioxidant capacity and HDL-cholesterol concentrations due to the presence of the flavonoids which have ability to bind with the lipoproteins [33]. From the results it can be concluded that the group V with 50 percent supplementation has reported a maximum decreasing effect on the blood plasma cholesterol levels. Therefore, it can be inferred that the present findings are in accordance with the literature report.

## Conclusion

Among the various dietary groups, dietary group with 50 percent dietary supplementation has resulted in maximum percent reduction in blood glucose (16.81 percent) and blood plasma cholesterol (33.94 percent) levels and 4.16 per cent increase in body weight. Consequently, the multifunctional ingredient approach in formulating the diabetic dietary supplement has opened up a new vista to combat both hyperglycemic and hypercholesterolemic tendencies in diabetic subject.

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## Conflict of Interest

The authors declare no conflict of interest.

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