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Evaluating the effect of functional foods supplementation to mitigate hyperglycemia

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Abstract

Functional and nutraceutical foods have emerged as a dietary intervention against various lifestyle-related disorders. Their nutritional aspects are primarily in several areas as cancer, cardiovascular disease (CVD), the aging process, diabetes and mental health. This research was conducted to analyze the effect of these oilseeds crops against different diseases and their compositional analysis was performed. To check their effect on human health study was conducted on rats in which effect on serum glucose, serum insulin, lipid profile including LDL, HDL, triglycerides, etc was determined. The effect of sesame on blood profile was studied by adding its specific proportion to rat feed and clinical tests were performed. Results declared that the clinical data consisting of 30 days study indicated that Seesame were found to be effective against diabtes. It was observed that cholesterol level, LDL, HDL were affected positively. Similarly, serum glucose and insulin indicated beneficial behavior in clinical tests. Conclusively, sesame and flaxseed powder because of their miracle properties were found best against cardiovascular diseases, obesity and many other diseases. T_2 (25% sesame and 75% flaxseed powder) indicated the best results against hyperglycemia.

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Introduction

Functional and nutraceutical foods have emerged as a dietary intervention against various lifestyle-related disorders. Their nutritional aspects are primarily in several areas as cancer, cardiovascular disease (CVD), the aging process, diabetes and mental health (Tewfik and Tewfik, 2008). The nutraceutical and functional foods made a positive impact on health as they contain bioactive components in products of interest (Shahidi and Naczk, 2004). This oil helps make cosmetics, insecticides and paints. Sesame seeds contain 50% to 58% of very good light-yellow crude oil characterized with the pleasing smell. This oil is stable to oxidative deterioration and does not rancid still after long contact to air. It also contains biologically active compounds like vitamin E mainly c-tocopherol. Vitamin E exists in eight structurally organized forms that consist of four tocopherol and four tocotrienols (Riaz *et al*., 2020).

The chemical composition of sesame seed depicts that it contains a valuable amount of oil (44-52%) and protein (18-23.5%). Besides, it also contains carbohydrates (13.5%), ash (5.3%) and moisture (5.2%). The protein is slightly low in lysine but rich in other amino acids especially leucine, cysteine, arginine and methionine. Sesame seeds contain good fat (i.e. monounsaturated fat 85%). It contains stearic acid (5-10%), oleic acid (35-43%). Sesame oil has a good flavor and regarded as better quality vegetable oil. It ranks second concerning nutritional value after olive oil. The composition of oil varies with the source and depends on factors like variety, soil type, climatic conditions and maturity of the plants (Kahyaoglu and Kaya, 2005).

Chinese utilized the oil extracted from sesame during the 4th century as a remedy for diseases of gums and toothaches. Oil of sesame seeds is well renewed to lower down the cholesterols because of its increased polyunsaturated fat volume other beneficial medicinal uses of sesame include treatment for headaches, dizziness and blurred vision. Research in the current era points out that antioxidant-rich foods help fight against heart-related problems and cancer. Therefore, plant driven antioxidants are of much concern. *Sesame indicum* L. is used for the savory purpose in Asian cooking (Tufail *et al*., 2018).

Several previous studies illustrated that dietary sesame lignans show beneficial properties like lowering serum cholesterol and level liver damage, enhancing α- tocopherol availability, vitamin E activities, and reducing thiobarbituric acid reactive substance (TBARS), which plays a role in the aging process by causing lipid peroxidation in membranes (Kato *et al*., 2005).

Sesaminol, another lignan present in sesame, inhibits unsaturated oil peroxidation in LDL (low- density lipoprotein) encouraged by 2,2Vazobis (2,4-dimethylvaleronitrile), (AMVN), $CuSO_4$, or 2,2V-azobis (2-amidinopropane) dihydrochloride (AAPH) in *vitro*. Data suggested that oxidative modification of LDL was successfully decreased by the presence of sesaminol. Hence, the recent data suggest that sesame supplementation might be a favorable adjunctive remedy in tackling hypercholesterolemia (Tufail *et al*., 2020).

The oil component shows significant stability to oxidation. It could be attributed to endogenous antioxidants which play an important role in trapping free radicals. It is also rich in tocopherols. Sesame oil mainly contains 50-373 ppm of α- tocopherols and 90-390 ppm of γ- tocopherols (Asghar *et al*., 2014). It also possesses 900-3000 ppm of phytosterols. β-sisterol, campesterol and signmasterol are the major phytosterols present in sesame oil. Sesame seed coat contains different polyphenols that include phenolic acids (Elleuch *et al*., 2007). Main objective of this study was to evaluate the effect of seasame and flaxseed agains hyperglycemia.

Materials and methods

In vivo studies

For efficacy trials, twenty rats were acquired from the University of Agriculture, Faisalabad and kept in Animal Room. At the start of the study, blood and serum samples were collected to get baseline values.

Classification of animals was done by dividing them equally into five groups. 4 rats were placed in each group. During the entire experimental period, the Animal Room was maintained at a temperature and relative humidity of 23±2°C and 55±5% respectively, with 12:12 hrs light: dark cycle. During 4 weeks of trial, normal, functional and nutraceutical diets were given to the respective groups under each study to evaluate the effects of individual treatment on the selected parameters including serum lipid profile, glucose and insulin levels. Animal diet efficacy plan in various groups is shown below in the Table 1.

Study I: Normal diet

For an initial period of one week, the basal diet was given to the rats to acclimatize them to the environment. Later, the diets containing flaxseed and sesame seed powder were provided. The experimental diet consisted of flour (82%), corn oil (10%), casein (4%), mineral mix (3%) and vitamin mix (1%).

Feed and drink intake was measured daily throughout the experimental period, while bodyweight weekly. At the termination of animal study, overnight fasted rats were sacrificed. Blood samples of rats were collected through a cardiac puncture in EDTA coated tubes for hematological study and non-coated tubes to measure serum lipid profile and glucose and insulin levels through Microlab-300, Merck, Germany.

Similarly, two other studies were also conducted to determine the impact of functional and nutraceutical foods against respective diets i.e. high cholesterol and high sucrose diet in separate rat modeling.

Study II: High sucrose diet

In study II, high sucrose diet containing 40% sucrose was given to the normal rats to determine the effect on serum glucose and insulin levels. Simultaneously, the effect of functional and nutraceutical diets on the induced trait in relevant groups of rats was assessed.

Study III: High cholesterol diet

In study III, high cholesterol diet containing 1.5% of cholesterol was fed to the normal rats to raise their lipid profile i.e. cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides. Afterward, flaxseed and sesame seed powder containing diets were provided to the respective rat groups at the same time to harmonize their effect on the respective category.

Feed and drink intake

The average feed intake of each group was measured daily by eliminating spilt diet from the total diet given during the whole study period (Wolf and Weisbrode, 2003). The water intake for each group was also recorded daily.

Bodyweight gain

The gain in body weight of experimental groups was measured weekly throughout the study period to monitor the effect of different diets on body weight gain.

Serum lipid profile

Serum lipid profiles of rats including cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LPL) and triglycerides were measured by following their respective protocols. Detail for each parameter is given below:

Cholesterol

The serum cholesterol level of rats was measured using CHOD–PAP method following the protocol of Kim *et al.* (2011).

High and low-density lipoprotein

High-density lipoprotein (HDL) and low-density lipoproteins (LDL) in serum samples were calculated by the method as mentioned by Kim *et al.* (2011).

Triglycerides

Triglycerides level in serum samples was estimated by the liquid triglycerides (GPO–PAP) method as illustrated by Kim *et al.* (2011).

Serum glucose and insulin levels

For each study, the collected serum was evaluated for glucose concentration by GOD-PAP method whereas

insulin level was assessed following the method of Kim *et al.* (2011).

Statistical analysis

The data for each parameter was subjected to statistical analysis to determine the level of significance by using SPSS version 21 and comparison of means was also carried out according to the method as described by (Montgomery, 2008).

Results and discussion

Serum profile

Serum glucose: Glucose is the carbohydrate normally found in mammalian blood and it is referred to as the "blood sugar." With few exceptions, it is equally distributed. The analysis of variance for serum glucose has been illustrated in Table 2 the data in the table indicates that in terms of days serum glucose was found to be affected significantly (P<0.05). In terms of treatment serum glucose was found to be affected significantly (P<0.05).

In terms of the interaction between days and treatment results were found to be nonsignificant (P>0.05). Interaction means for days and treatment have been illustrated in Table 2. The data in the table indicates that values ranged from 116.67 to 126.33 (mg/dL). The lowest mean value was observed in the case of T_3 at 15 days (116.67 (mg/dL)) and the highest value was observed in the case of T_4 at 30 days (126.33 (mg/dL)). In terms of overall means, the highest value was observed in T_4 which was 122.44 and T_4 at 30 days contributed maximum to that. Our results were found similar to the Ford *et al.* (2001). Butt *et al.* (2007) also concluded that increasing the level of soluble fiber of guar gum, chickpea, and lentil in chapattis contributed towards a decrease in serum glucose concentration of Sprague Dawley rats.

Table 1. Animal diet efficacy plan.

Group	Description
G_1	Normal diet
G_2	Hyperlipidemic control (Normal Diet)
G_3	Sesame seed powder 10% of normal diet
G_4	Flaxseed powder 10% of normal diet
G_5	Sesame seed and Flaxseed powder 10% of normal die diet in 1:1

Serum insulin: Insulin helps to transport glucose from the blood to within cells, thus helping regulate blood glucose levels, and has a role in lipid metabolism. The data in the table indicates that in terms of days serum insulin was found to be effected none significantly (P>0.05). Interaction means for days and treatment have been illustrated in Table 3.

Table 2. Days × Treatment interaction Means for Serum Glucose (mg/dL) of Rat groups.

Sample Type	Days			Mean
	0	15	30	
To	120.0±1[a]	116.67±2.08[a]	117.33±4.16[a]	118.0[a]
T_1	125.0±1.73[a]	118.67±3.05[a]	117.0±1[a]	122.22[a]
T_2	124.67±2.08[a]	119.0±4[a]	123.0±7[a]	122.22[a]
T_3	125.67±2.88[a]	116.67±1.52[a]	124.33±1.15[a]	122.22[a]
T_4	117.0±7[a]	124.0±6.08[a]	126.33±0.57[a]	122.44[a]
Mean	122.47[a]	119.0[b]	121.60a[b]	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

The data in the Table indicates that values ranged from 4.63 to 5.86 (µU/mL). The lowest mean value was observed in the case of To at 0 days (4.63 (µU/mL)) and the highest value was observed in the case of T_2 at 15 days (5.86 (µU/mL)). In terms of overall means the highest value was observed in T_4

which was 122.44 and T_4 at 30 days contributed maximum to that. Our results were found similar to the Ford *et al.* (2001).

Lipid profile

Cholesterol: The data indicates that cholesterol was found to be affected highly significantly (P<0.01). In terms of treatment, cholesterol was found to be affected highly significantly (P<0.01). In terms of the interaction between both results were found to be highly significant (P<0.01). Interaction means for days and treatment have been illustrated in Table 4. The data in the table indicates that values ranged from 96.67 to 176.33 (mg/dL).

Table 3. Days × Treatment interaction Means for Serum Insulin (µU/mL) of Rat groups.

Sample Type	Days			Mean
	0	15	30	
T_0	4.63±0.55^a	5.62±0.19^a	5.67±0.58^a	5.30^a
T_1	4.66±0.87^a	5.78±0.07^a	5.78±0.34^a	5.434^a
T_2	5.38±0.63^a	5.86±0.06^a	5.85±0.16^a	5.78^a
T_3	5.13±0.16^a	5.38±0.24^a	5.79±0.07^a	5.43^a
T_4	5.24±0.47^a	5.74±0.24^a	5.70±0.31^a	5.56^a
Mean	5.009^a	44.50^a	5.76^a	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

The lowest mean value was observed in the case of T_3 at 0 days (96.67 (mg/dL)) and the highest value was observed in the case of T_3 at 15 days (176.33 (mg/dL)). In terms of overall means, the highest value was observed in T_1 which was 149.11 and T_1 at 30 days contributed maximum to that. Our results were found similar to Juan *et al.* (2005).

Table 4. Days × Treatment interaction Means (mg/dL) for Cholesterol of Rat groups.

Sample Type	Days			Mean
	0	15	30	
T_0	98.33±0.57^c	97.67±1.52^c	97.00±1.00^c	97.67^c
T_1	97.00±1.00^c	174.33±1.53^a	176.00±2.00^a	149.11^a
T_2	98.00±1.00^c	175.67±2.52^a	154.00±1.00^b	142.56^b
T_3	96.67±1.15^c	176.33±2.52^a	153.67±1.15^b	142.22^b
T_4	98.00±1.00^c	174.67±2.51^a	156.00±1.00^b	142.89^b
Mean	97.60^c	159.73^a	147.33^b	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

Table 5. Days × Treatment interaction Means for LDL (mg/dL) of Rat groups.

Sample Type	Days			Mean
	0	15	30	
To	62.00±1^d	61.33±3.05^d	61.00±2^d	61.44^c
T_1	59.00±1^d	103.00±4^a	103.00±2^a	88.33^a
T_2	61.67±1.52^d	105.67±1.52^a	71.00±2^b	79.44^b
T_3	59.33±1.52^d	105.00±2.64^a	71.33±3.05^b	78.56^b
T_4	63.00±2^{cd}	105.67±2.30^a	63.00±1.52^{cd}	79.33^b
Mean	61.00^c	96.13^a	75.13^b	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

LDL: The data in the table indicates that in terms of days LDL was found to be affected highly significantly (P<0.01). In terms of treatment, LDL was found to be affected highly significantly (P<0.01). In terms of the interaction between days and treatment results were found to be highly significant (P<0.01). Interaction means for days and treatment have been illustrated in Table 5. The data in the table indicates that values ranged from 59.00 to 105.67 (mg/dL). The lowest mean value was observed in the case of T_1 at 0 days (59.00 (mg/dL)) and the highest value was observed in the case of T_4 at 15 days (105.67 (mg/dL)).

The highest value was observed in T_1 which was 88.33 and T_1 at 15 days contributed maximum to that. Our results were found similar to the Ford *et al.* (2001).

Table 6. Days × Treatment interaction Means for HDL of Rat groups.

Sample Type	Days			Mean
	0	15	30	
To	45.33±1.52^{b}	46.00±3^{b}	47.33±1.52^{ab}	46.22^{a}
T_1	46.00±2^{b}	39.00±1^{c}	37.00±1^{c}	40.67^{c}
T_2	48.00±2^{ab}	37.00±1^{c}	40.67±0.57^{c}	41.89^{bc}
T_3	47.00±1^{b}	37.67±1.52^{c}	40.33±1.52^{c}	41.67^{bc}
T_4	51.67±1.52^{a}	37.00±1^{c}	40.00±1^{c}	42.89^{b}
Mean	47.60^{a}	39.33^{c}	41.07^{b}	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

HDL: The data in the table indicates that in terms of days HDL was found to be affected highly significantly (P<0.01). In terms of treatment, HDL was found to be affected highly significantly (P<0.01). In terms of the interaction between days and treatment results were highly significant (P<0.01). Interaction means for days and treatment have been illustrated in Table 6. The data in the table indicates that values ranged from 37.00 to 51.67 (mg/dL). The lowest mean value was observed in the case of T_1 at 30 days (37.00 (mg/dL)) and the highest value was of T_4 at 0 days (51.67 (mg/dL)). In terms of overall means, the highest value was observed in T_0 which was 46.22 and T_0 at 30 days contributed maximum to that. Our results were found similar to the Ford *et al.* (2001).

Table 7. Day's × Treatment interaction means for Triglycerides of Rat groups.

Sample Type	Days			Mean
	0	15	30	
To	71.00±2^{d}	74.00±3.60^{d}	71.00±3^{d}	72.00^{b}
T_1	71.67±1.52^{d}	139.00±9.64^{a}	105.67±2.08^{b}	105.44^{a}
T_2	70.33±1.15^{d}	145.33±5.13^{a}	93.33±1.52^{c}	103.00^{a}
T_3	69.33±3.21^{d}	145.67±4.93^{a}	90.00±1^{c}	101.67^{a}
T_4	70.33±1.52^{d}	148.67±4.04^{a}	87.00±2^{c}	102.00^{a}
Mean	70.53^{c}	130.53^{a}	89.40^{b}	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

Triglycerides**:** A triglyceride is an ester derived from glycerol and three fatty acids. Triglycerides are the main constituents of body fat in humans and other vertebrates, as well as vegetable fat. The data in the table indicates that in terms of day's triglyceride was found to be affected highly significantly (P<0.01). In terms of treatment, triglyceride was found to be affected highly significantly (P<0.01). In terms of the

interaction between days and treatment results were found to be highly significant (P<0.01). Interaction means for days and treatment have been illustrated in Table 7. The data in the table indicates that values ranged 69.33 from to 148.67. The lowest mean value was observed in the case of T_3 at 0 days (69.33) and the highest value was observed in the case of T_4 at 15 days (148.67). In terms of overall means, the highest value was observed in T_1 which was 105.44 and T_1 at 30 days contributed maximum to that. Our results were found similar to the Duan *et al.* (2003).

Table 8. Week × Treatment interaction Means (mg/dL) for Weight of Rat groups.

Sample Type	Week				Mean
	1	2	3	4	
To	178.92±3^{a}	173.87±55.43^{a}	171.95±2^{a}	177.32±1.52^{a}	187.08^{a}
T_1	187.33±2.51^{a}	197.33±3.51^{a}	195.33±2.08^{a}	195.67±1.52^{a}	193.92^{a}
T_2	191.00±2.64^{a}	192.67±3.51^{a}	187.67±4.50^{a}	184.00±4.58^{a}	188.83^{a}
T_3	183.67±2.51^{a}	176.67±2.51^{a}	184.67±4.04^{a}	183.33±2.08^{a}	182.08^{a}
T_4	185.67±3.51^{a}	188.00±5^{a}	180.67±1.52^{a}	193.34±5.03^{a}	183.25^{a}
Mean	188.33^{a}	184.33^{a}	188.67^{a}	186.80^{a}	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

Table 9. Week × Treatment interaction Means for Feed of Rat groups.

Sample Type	Week				Mean
	1	2	3	4	
To	19.74±1.55^{b}	20.33±0.50^{ab}	20.37±0.93^{ab}	19.87±0.73^{ab}	20.08^{ab}
T_1	21.24±0.92^{ab}	20.23±0.32^{ab}	22.60±0.46^{a}	20.24±0.41^{ab}	21.08^{a}
T_2	20.13±1.01^{ab}	19.64±0.35^{b}	20.24±0.63^{ab}	19.83±1.09^{ab}	19.96^{b}
T_3	20.69±0.47^{ab}	21.21±0.67^{ab}	20.71±1.30^{ab}	19.95±1.52^{ab}	20.64^{ab}
T_4	20.75±0.98^{ab}	21.17±0.96^{ab}	20.63±0.75^{ab}	20.16±0.97^{ab}	20.68^{ab}
Mean	20.51^{ab}	20.52^{ab}	20.91^{a}	20.01^{b}	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

Body Weight of rats

The data in the table indicates that in term of weight of rats was found to be effected none significantly (P>0.05). In terms of the treatment weight of rats was found to be effected none significantly (P>0.05). In terms of the interaction between days and treatment results were found to be non-significantly (P>0.05). Interaction means for days and treatment have been illustrated in Table 8. The data in the table indicates that values ranged from 171.95 to 197.33. The lowest mean value was observed in the case of To at 3 weeks (171.95 g) and the highest value was observed in the case of T_1 at 2 weeks 19197.33 g). In terms of overall means, the highest value was observed in T_1 which was 193.92 and T_1 at 2 weeks contributed maximum to that. Our results were found similar to the Duan *et al.* (2003).Visavadiya *et al.* (2009) also observed the same results in their research.

Table 10. Week × Treatment interaction Means for the water of Rat groups.

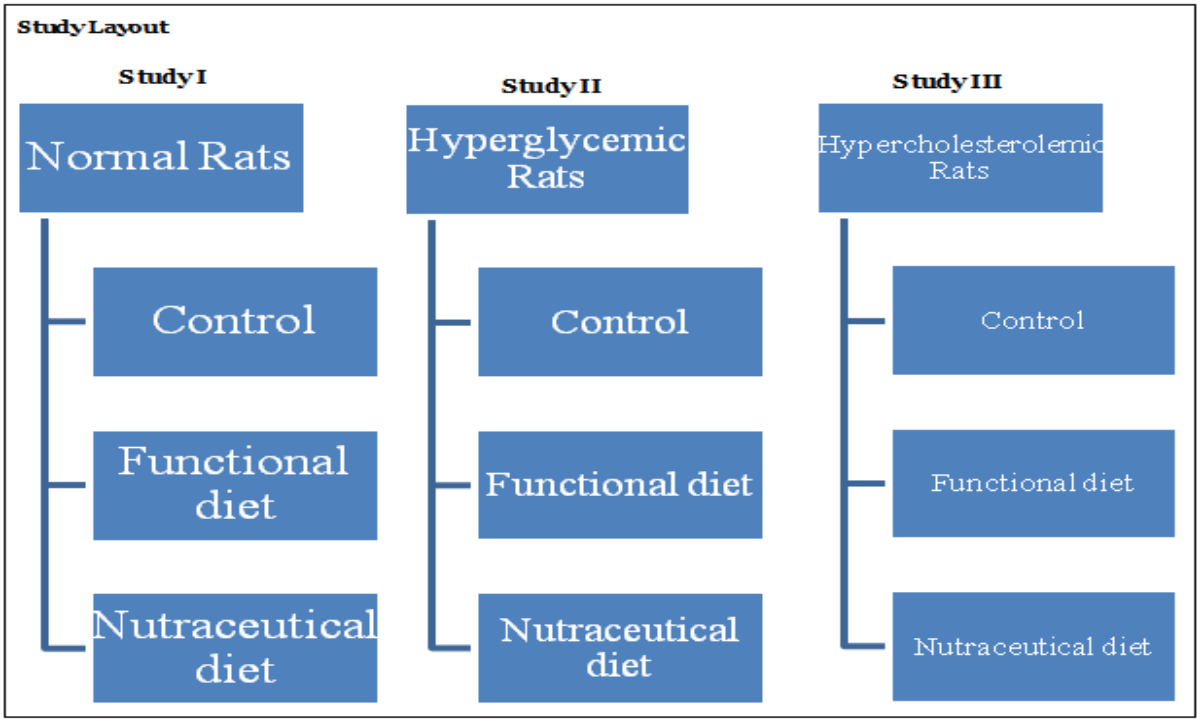
Sample Type	Week				Mean
	1	2	3	4	
To	244.00±3^{c}	252.00±9.53^{abc}	243.33±0.57^{c}	245.00±1^{c}	246.08^{ab}
T_1	253.0±3.46^{abc}	24700±2.64^{abc}	256.0±2^{ab}	243.67±3.05^{c}	249.92^{a}
T_2	245.33±3.05^{bc}	257.0±1^{a}	244.67±3.51^{c}	244.67±3.05^{c}	247.92^{ab}
T_3	252.67±3.51^{abc}	244.33±3.05^{c}	245.00±3.60^{c}	246.33±3.05^{abc}	247.08^{ab}
T_4	244.67±4.04^{c}	244.67±3.51^{c}	246.33±3.05^{abc}	246.00±2^{bc}	245.42^{b}
Mean	247.93^{ab}	249.0^{a}	247.07^{ab}	245.13^{b}	

The difference among means shows a significant effect (P<0.05). Small letters represent comparison among interaction means and overall means.

They observed a dose-dependent effect of sesame seed powder on hypercholesterolemia and oxidative stress condition in albino rats. In this study, they also observed the body weight and liver weight of rats before and after study.

Feed Intake of rats

The data in the table indicates that in term of feed of rats was found to be affected significantly ($P<0.05$). In term of treatment feed of rats was found to be affected none significantly ($P>0.05$). In terms of the interaction between days and treatment results were found to be nonsignificant ($P<0.05$). Interaction means for days and treatment have been illustrated in Table 9. The data in the table indicates that values ranged from 19.74 to 22.60 g. The lowest mean value was observed in the case of T_0 at 1 week 19.74 g) and the highest value was observed in the case of T_1 at 3 weeks (22.60 g). In terms of overall means, the highest value was observed in T_1 which was 21.08 and T_1 at 3 weeks contributed maximum to that. The above results are in comparison with Visavadiya *et al.* (2009). In their study, they examined the hypocholesterolemic activity and antioxidative potential of sesame seeds. Bodyweight feed and drink intake of rats were also examined. Their results suggested that no significant differences were observed between the feed intakes of all the groups.



Control : Normal diet
Functional diet : Diet containing flaxseed powder
Nutraceuticals diet : Diet containing sesame extracts

Fig. 1. Different groups conducted in the efficacy trials.

Water Intake of rats

The data in the table indicates that in terms of day's water intake of rats was found to be affected significantly ($P<0.05$). In terms of treatment, water intake was found to be affected significantly ($P<0.05$). In terms of the interaction between days and treatment results were found to be nonsignificant ($P>0.05$). Interaction means for days and treatments have been illustrated in Table 10. The data in the table indicates that values ranged from 243.33 to 257.0 ml. The lowest mean value was observed in the case of T_0 at 3 weeks (243.33 ml) and the highest value was observed in the case of T_2 at 2 weeks (257.0 ml). In terms of overall means, the highest value was observed in T_1 which was 249.92 and T_1 at 3 weeks contributed maximum to that.

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