



Supplementation of Seed Dust of *Vicia Faba* and Sesame Ameliorates High Lipid Diet-Induced Dyslipidemia in Rats

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Abstract

Dyslipidaemia is a life style disorder characterized by increased levels of total cholesterol, LDL cholesterol and triglycerides and also decreased HDL cholesterol levels which is turned into cardiovascular disease, a first leading cause of death in Worldwide. Lifestyle changes mainly healthy diet should be introduced to reduce the cardiovascular risk. Recent research on functional foods consumption for lipid-lowering effects has been well established. Seeds of *Vicia faba* (SVf) and sesame seeds (SSi) is one of the most popular foods due to high content of dietary fiber, proteins, vitamins, minerals, phytosterol, omega 3 fatty acids and other functional compounds. The study aimed to establish SVf and SSi as a powerful functional food for prevention and management of dyslipidemia. We formulated a normal diet (ND) and high lipid diet (HLD) for rats. HLD was formulated by increasing the 10.48%energy, 60.07%lipid, 100%cholesterol and 50% sucrose than ND. Rats were randomly divided into five groups fed ND, HLD, HLD+10%SVf dust, HLD+10%SSi dust and HLD + 5% SVf + 5% SSi of total food for 60 days. After 60 days of treatment, it was observed that there was a significant ($p < 0.05$) increase in plasma triglyceride, total cholesterol, LDL-C, malondialdehyde (MDA) and IL-18 levels but a significant ($p < 0.05$) decrease in HDL-C, super oxide dismutase (SOD) & catalase activity in HLD in compared with ND and other treated groups. HLD induced dyslipidemia while SVf and SSi produced antidyslipidemic activity decreasing plasma triglyceride, total cholesterol, LDL-C, MDA, IL-18 level and increasing HDL-C, SOD, and catalase. SVf and SSi combined feeding was potential synergistic effect and results suggest that this functional food consumption can prevent and management of dyslipidemia.



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Introduction

Beans are the edible seed portion of family fabaceae of leguminous plants including various variety like *Vicia*, *Vigna*, *Cicer*, *Lathyrus* which are consumed by animals and human to meet ideal nutritional demand like high protein,¹ dietary fiber,² low fat, good source of vitamins and minerals³ and phytochemicals including total phenolics, total flavonoid, tannin, and phytosterol.⁴ *Vicia Faba* contains a good proportion of nutrients like high protein, dietary fiber, folic acid and vitamins⁵. Regular beans consumption may improve glycemic control,^{6,7} reduce cholesterol level and coronary heart diseases, prevent colon cancer and control diabetes,⁶⁻⁸ slow down the risk of obesity⁹ and antioxidant and anti-inflammatory effect. Sesame (*Sesamum indicum* L.) belongs to the family *Pedaliaceae*, which contains moisture, crude proteins, carbohydrates, crude fiber, and ash¹⁰ oil (44–58%), mono and poly unsaturated fatty acid (oleic acid and linoleic acid)¹¹⁻¹³ various types of vitamins and minerals,¹⁴ two lignans like sesamol, sesamolinal, pinosresinol, sesaminol and phytosterol.¹⁵ Sesame seed has a positive influence on the antioxidant level,¹⁶ lipid peroxidation,¹⁷ plasma cholesterol and triacylglycerol level, blood glucose level and blood pressure.¹⁸⁻²⁰ Hyperlipidemia is one of the most risk factors for cardiovascular diseases (CVD) which is now first leading cause of death in World.²¹ Prevention and management of this disease is now the first priority of the research field among scientists in the world. Experimental studied showed that *Vicia Faba* and sesame seeds in the diet of rat model improved the condition of hyperlipidemia.^{6,20} NCEPP (National Cholesterol Education Prevention Programme) recommended 2 g plant sterol in the daily diet reduces the plasma cholesterol by 10% in one month.²² However, to the best of our knowledge, no study has been explored for supplementation of seeds of *Vicia Faba* (SVf) and sesame seeds (SSi) separately or combined in the diet as a therapeutic role against secondary hyperlipidemia in rats. Therefore, the aim of the present study was to evaluate the effect of individual supplementation of SVf and SSi and their composite manner for the studied period of 60 days on plasma lipid biomarkers, atherogenic index, oxidative stress, liver function enzymes, antioxidant enzymes and IL-18 level of secondary hyperlipidemia in rats.

Materials and Methods

Preparation of Seed of SVf and SSi Dust

The plant materials for the experiment consist of fresh broad bean (*Vicia Faba*) and Sesame (*Sesamum indicum*) seeds. The broad beans were harvested at the kitchen garden of the Department of Nutrition, Raja Narendra Lal Khan Women's college, Midnapore, West Bengal, India. Sesame seeds were purchased from a local fruit market in Midnapore, India. Then *Vicia Faba* were washed well and separated from the seeds. Seeds were dried at 40±1°C in an incubator for 3 days. Then both dried seeds were crushed separately in an electric grinder machine. After that, the fine dust of SVf and SSi were considered for the experimental study.

Selection of Animals and Care (Experimental Subjects and Model)

The present experiment was conducted on 30 male *Wistar* strain adult pathogen free and healthy albino rats having the weight of 250±15 g (rats were purchased from Saha Enterprise (Laboratory Animal Supplier), 386/2, Nilachal, Birati, Kolkata-700051, CPCSEA registered under Ministry of Environment and Forest, Government of India vide no. 1828/PO/BT/S/15/CPCSEA). Animals were kept in normal laboratory condition for 2 weeks prior to experimentation. The animals were housed with 3 rats/cage in a temperature-controlled room (22±20 °C) with 12-12 h dark-light cycles (8.00-20.00 h light, 20.00-8.00 h dark) at a humidity of 50 ± 10%. They were provided with standard food and water ad libitum. Animal care was provided according to the Guiding Principle for the Care and Use of Animals.²³ The Institutional Animal Ethical Committee (Registration number: 1905/PO/Re/S/2016/CPCSEA) approved this study to use only 30 rats.²⁴ ND (Normal diet) rats fed experimental diet according to AIN-93G with minor modification. Induction of dyslipidemia was carried out on HLD, HLD + SVf, HLD + SSi and HLD + SVf + SSi by addition of extra 11g coconut oil, 0.5% cholesterol and 5g sucrose in normal diet. HLD + SVf rats was supplemented SVf 10%, HLD + SSi rats fed SSi 10%, and HLD + SVf + SSi rats fed SVf 5% + SSi 5% of total foods. (Table 1). The Strategy of combined supplementation keeping view in mind that SVf contain high amount of protein, low in fat, low in phytosterol and SSi contain high amount of phytosterol and unsaturated fat.

Reagents and Chemicals

Major biochemical parameters like total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), glutamic oxalic transaminase (GOT), glutamate pyruvate transaminase (GPT) were measured by Semi-autoanalyser, other biochemical parameters like Superoxide Dismutase (SOD), Catalase (CAT) and malondialdehyde (MDA) were measured by absorbance UV-VIS Spectrophotometer.

Preparation of the Samples for Biochemical Studies

This experimental study was continued for 60th days. On the 61st day of the experiment, those animals were sacrificed under anesthesia and blood was collected from the aorta and plasma was collected by centrifuge tube. Liver, kidney tissues were collected for determination of different biochemical parameters.

Estimation of Plasma Lipid Profile

TG, TC, HDL-C, and LDL-C were estimated according to our earlier study²⁵ by semiauto analyzer using the standard kit method and the values are expressed as mg/dL.

Fecal Cholesterol Analysis

Feces were collected for 7 days before and at the end of 60days, lyophilized, and then milled into a powder. Total lipids were extracted with chloroform: methanol (2:1 v/v) according to the method of Folch *et al.*²⁶

Measurement of Antioxidant Enzyme Profiles

The SOD activity was estimated as described by Pradhan *et al.*²⁷ and values were expressed as nmol of H₂O₂ consumption/mg of tissue/min. CAT activity was assayed according to the method of Roy *et al.*²⁸ and values were expressed as nmol of H₂O₂ consumption/mg of tissue/min.

Quantification of MDA Level

The level of MDA was determined by the established method of Das *et al.*²⁹ in liver tissue which was measured by spectrophotometer at 535 nm and values are expressed nmol/mg of tissue.

Toxicity Profile Marker of Plasma and Liver Tissue

Liver tissues were homogenized separately in 0.05 M tris-hydrochloric acid (HCl) buffer solution (pH

7.0) at the tissue concentration of 50 mg/mL. This homogenate was centrifuged separately at 10,000 g at 4 °C for 10 min and tissue supernatant was collected. Activities of liver enzymes as GOT, GPT were estimated by the method of Roy *et al.*³⁰

Determination of IL 18 Level using ELISA

The cells were seeded into 96 well plates with 300 µl plasma, containing 2x10⁶ MPMs/mL. The supernatant was stored at -20°C and the levels of IL-18 measured using a specific rat IL-18³¹ as per information provided in the kit manual (Invitrogen Bioservices India Pvt. Ltd).

Histopathological Analysis of Liver Tissue

Liver tissues from the experimental rats were fixed in 10%buffered formalin solution embedded in paraffin wax and 5 µm sections were prepared with a rotary microtome. These thin sections were stained with hematoxylin and eosin (H and E), mounted on glass slides and observed for pathological changes under a binocular microscope according to Mani.³²

Data and Statistical Analysis

Data are expressed as a mean ± standard error (SE; (n= 6). ANOVA followed by Bonferroni t-test to detect intergroup differences multiple two-tail t-test and bars differ from each other significantly (p < 0.05).³³ Correlations between quantitative normally distributed parameters were assessed with Pearson's two-way test. A p-value less than 0.05 was considered significant.

Results and Discussion

From the various literatures on dietary management of dyslipidemia in various animal models followed by only cholesterol supplemented to diet with modified in lipids, carbohydrate and sucrose contents.³⁴⁻³⁶ A model diet for induction of dyslipidemia in rat model based on the increased energy, cholesterol, fat, saturated fatty acids, PUFA, MUFA, dietary fiber and phytosterol intake was planned. HLD group had increased energy, fat, cholesterol, sucrose by 10.48%, 60.07%, 100%, 50% respectively than the ND group. In the second HLD + SVf group had increase energy, fat, cholesterol, sucrose by 10.06%, 60.48%, 100%, 50% respectively than the ND group. In third HLD+SSi group had increase energy, fat, cholesterol, sucrose by 15.35%, 70.80%, 100%, 50% respectively than the ND group. In forth, HLD

Table 1: Composition of the experimental diet and its nutritional analysis

| Foods(g) | ND (n=6) | HLD (n=6) | HLD +SVf (n=6) | HLD + SSi (n=6) | HLD +SVf + SSi (n=6) |
|----------------------|---------------------|----------------------|---------------------------|----------------------------|---------------------------------|
| Corn Starch(g) | 40 | 40 | 30 | 30 | 30 |
| Casin(g) | 25 | 20 | 20 | 20 | 20 |
| Bengal gram flour(g) | 16 | 15 | 15 | 15 | 15 |
| Milk powder(g) | 10 | - | - | - | - |
| Sucrose(g) | 5 | 10 | 10 | 10 | 10 |
| Coconut Oil(g) | 2 | 13 | 13 | 13 | 13 |
| Mineral Mixture(g) | 1 | 0.5 | 0.5 | 0.5 | 0.5 |
| Cholesterol(g) | - | 0.5 | 0.5 | 0.5 | 0.5 |
| Vitamin Premix(g) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Salt(g) | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| SVf(g) | - | - | 10 | - | 5 |
| SSi(g) | - | - | - | 10 | 5 |
| Total | 100 | 100 | 100 | 100 | 100 |
| Energy(kcal) | 382.19 | 426.98 | 425.48 | 451.54 | 438.96 |
| Carbohydrate(g) | 59.44 | 58.26 | 54.99 | 51.5 | 53.24 |
| Protein(g) | 23.98 | 18.11 | 20.69 | 19.85 | 20.27 |
| Fat(g) | 5.39 | 13.5 | 13.64 | 18.46 | 16.05 |
| SFA(g) | 3.48 | 10.73 | 10.76 | 11.43 | 11.09 |
| PUFA(g) | 0.56 | 0.88 | 0.94 | 3.06 | 2.0 |
| MUFA(g) | 0.53 | 1.16 | 1.19 | 3.04 | 2.12 |
| Dietary Fiber(g) | 3.08 | 2.91 | 5.32 | 4.0 | 4.66 |

Table 2: Food intake, fluid intake by each rat calculated by average intake from six rats

| | ND | HLD | HLD+SVf | HLD+SSi | HLD+SVf+SSi |
|---------------------------------|-----------|------------|----------------|----------------|--------------------|
| Diet and Water | ND+ Water | HLD+ Water | HLD+ SVf | HLD+ SSi | HLD+SVf +SSi |
| Food intake (g/day) | 15.05 | 16.99 | 15.87 | 14.81 | 15.13 |
| Fluid Intake (mL/day) | 25.2 | 26.5 | 24.9 | 25.1 | 25.8 |
| Energy Intake(Kcal/rat/day) | 57.51 | 72.54 | 67.52 | 66.87 | 66.41 |
| Carbohydrate Intake(g/rat/day) | 8.94 | 9.89 | 8.72 | 7.62 | 8.05 |
| Protein Intake(g/rat/day) | 3.60 | 3.07 | 3.28 | 2.93 | 3.06 |
| Fat Intake(g/rat/day) | 0.811 | 2.293 | 2.164 | 2.73 | 2.42 |
| SFA Intake(g/rat/day) | 0.326 | 1.82 | 1.70 | 1.69 | 1.67 |
| PUFA Intake(g/rat/day) | 0.084 | 0.149 | 0.149 | 0.453 | 0.302 |
| MUFA Intake(g/rat/day) | 0.079 | 0.197 | 0.188 | 0.450 | 0.320 |
| Dietary Fiber Intake(g/rat/day) | 0.463 | 0.494 | 0.844 | 0.592 | 0.705 |

ND : Normal Diet ; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba ; HLD+SSi: High Lipid Diet + Sesame seeds; HLD+SVf+SSi : High Lipid Diet+ Seed of Vicia faba + Sesame seeds; SFA: Saturated Fatty Acid; PUFA: Polyunsaturated Fatty Acid; MUFA: Monounsaturated Fatty Acid

+ SVf + SSi group had increase energy value, fat, cholesterol, sucrose by 12.93%, 66.41%, 100%, 50% respectively than the ND group.

Body Weight, Hepatosomatic Index and Atherogenic Index

Results of the present study revealed that body weight was significantly ($p < 0.05$) increased at the end of the experiment in HLD fed animals in comparison to ND group. The gain in body weight was significantly lower ($p < 0.05$) in three dietary intervention groups when compared to HLD fed animals. In relation to relative weights of liver there were significant ($p < 0.05$) increase in liver weight in HLD fed rats as compared to the ND group. High lipid diet (HLD) was significantly ($p < 0.05$) increased the mean body weight throughout the experimental period compared with animals fed SVf, SSi, SVf + SSi along with HLD by 38.51%, 30.26%, 23.87% and 27.27% respectively. Among the 3 dietary treatment groups, rats fed HLD + SVf had the highest weight gain, whereas the lowest weight gain was seen in rats fed HLD+SSi, these values become more similar to the ND animals (Table 3). It was observed the significantly ($p < 0.05$) decrease in the hepatosomatic index (H.S.I) and atherogenic index of plasma (AIP) values in HLD + SVf, HLD + SSi, HLD + SVf + SSi groups compared to HLD group. Among 3 dietary treatment groups, group HLD + SVf + SSi having most effective (Table

3). These may result due to the accumulation of fat in the liver cells leading to an increase in their weight. Histopathological examination of liver tissue showed the presence of fatty changes of hepatocytes of HLD group is compared with 3 dietary treatment groups. The results were agreed with those of Teresa Macarulla *et al.*³⁴ who reported that *Vicia Faba* when given to rats it decreased the liver weight. The study also revealed improvement in atherogenic index of plasma as a result of dietary interventions with HLD+SSi which contribute to cardioprotection.³⁷

Status of Lipid Profile, Lipidperoxidation and Pro-Inflammatory Marker During Experiment

It was demonstrated 26.99% elevation in circulating TG, 40.87% elevation in TC, 38.83% elevation in LDL, 39.77% elevation in fecal total lipid, 78.62% elevation in fecal cholesterol and 24.67% reduction in HDL of HLD in respect to ND. SVf 10% supplementation significantly ($p < 0.05$) depleted 11.36% in plasmatic TG, 14.14% depleted in TC, 19.34% depleted in LDL and 10.10% elevated in HDL, 42.57% elevated in fecal total lipid, 13.97% elevated in fecal cholesterol than the HLD. It is also shown that 23.51% decrement in TG, 32.79% decrement in TC, 39.27% decrement in LDL and 27.51% increment in HDL, 19.03% increment in fecal total lipid, 40.08% increment in fecal cholesterol in 10% SSi supplementation along with HLD in comparison to HLD. In supplementation with 5% SVf

Table 3: Influence of dietary SVf and SSi on body weight, liver weight, H.S.I and AIP in high lipid fed male albino rats. Data are expressed as mean \pm SE (n= 6). ANOVA followed by multiple two-tail t-test.0.05).

| | ND | HLD | HLD + SVf | HLD + SSi | HLD + SVf + SSi |
|--|-------------------|--------------------|--------------------|--------------------|-------------------|
| Initial Body weight(g) | 249.0 \pm 1.6 | 251.5 \pm 1.73 | 254.56 \pm 0.49 | 253.26 \pm 0.715 | 252.56 \pm 0.66 |
| Final body weight(g) | 364 \pm 1.2a | 406.33 \pm 1.28b | 362.83 \pm 1.24a | 335.2 \pm 1.87c | 342.5 \pm 2.81d |
| Percentage(%) of body weight increased | 31.48 | 38.15 | 30.26 | 23.87 | 27.27 |
| Total Liver weight(g) | 12.04 \pm 0.01a | 18.15 \pm 0.01b | 15.35 \pm 0.55c | 14.08 \pm 0.04d | 13.07 \pm 0.29e |
| Hepatosomatic Index | 3.311 | 4.461 | 4.221 | 4.078 | 3.913 |
| Atherogenic Index | 0.514 | 0.773 | 0.679 | 0.551 | 0.617 |

*Atherogenic Index of plasma (AIP) is calculated according to the formula, $\log(\text{TG}/\text{HDL}-\text{C})$

*Hepatosomatic Index (H.S.I) = (Weight of the liver/Weight of the Body)*100

ND : Normal Diet ; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of *Vicia faba* ; HLD+SSi: High Lipid Diet + Sesame seeds; HLD+SVf+SSi : High Lipid Diet+ Seed of *Vicia faba* + Sesame seeds.

+ 5% SSi significantly ($p < 0.05$) 17.91% diminution in TG, 27.16% diminution in TC, 31.40% diminution in LDL and 17.55% uplift in HDL, 31.46% uplift in fecal total lipid, 18.76% uplift in fecal cholesterol in respect to HLD. Evidence suggested that 10% SSi supplementation significantly ($p < 0.05$) reduced plasmatic TG, TC, LDL and induced the level of HDL, fecal total lipid, fecal cholesterol than the 10% SVf and combined supplementation of SVf and SSi (5%+5%) (Table 4).

Plasma CAT activity significantly ($p < 0.05$) 55.10% decreased and liver tissue CAT activity significantly ($p < 0.05$) 31.79% decreased in HLD with respect to ND. Supplementation with HLD + SVf was found to significantly ($p < 0.05$) increase the activity of CAT in 104.54% in plasma and 26.81% in liver tissue than the HLD. SSi supplementation 59.09% escalation in plasma and 12.68% escalation in liver CAT as compared to HLD. Combined supplementation of SVf + SSi significantly 77.27% raise in plasma and 16.78% raise in liver CAT level when compared to HLD. From the above result supplementation of 10% SVf significantly enhance the both plasma and liver CAT concentration than 10% SVf and SVf + SSi (Fig. 1).

In the animals that received HLD significantly ($p < 0.05$) reduced 55.90% SOD in plasma and 29.85% SOD in liver when compared to ND. In the same way, the group treated with 10% SVf significantly ($p < 0.05$) uplift 114.31% SOD in plasma and 33.47% SOD in liver tissue in respect to HLD. Likewise, 10% SSi treated rats presented elevation in 56.33% SOD in plasma and 14.46% SOD in liver homogenates than the HLD. The treatment with combined supplementation of SVf + SSi (5%+5%) significant increase 99.29% plasma SOD and 26.85% liver SOD when compared to the HLD. From the above result, 10% SVf showed an expressive elevation of SOD level in plasma and liver than the other two supplemented groups (Fig. 2).

It was observed the level of plasma and liver MDA significantly increased 50.49% and 26.40% in HLD than the ND. By the treatment with 10% SVf plasma and liver MDA concentration significantly ($p < 0.05$) lowered 21.73% and 9.18% respectively in comparison to HLD. Similarly, 10% SSi supplementation significantly ($p < 0.05$) downgrade the 56.84% MDA in plasma and 29.17% MDA in liver in respect to HLD. The concentration of MDA significantly ($p < 0.05$) depleted 42.80% in plasma

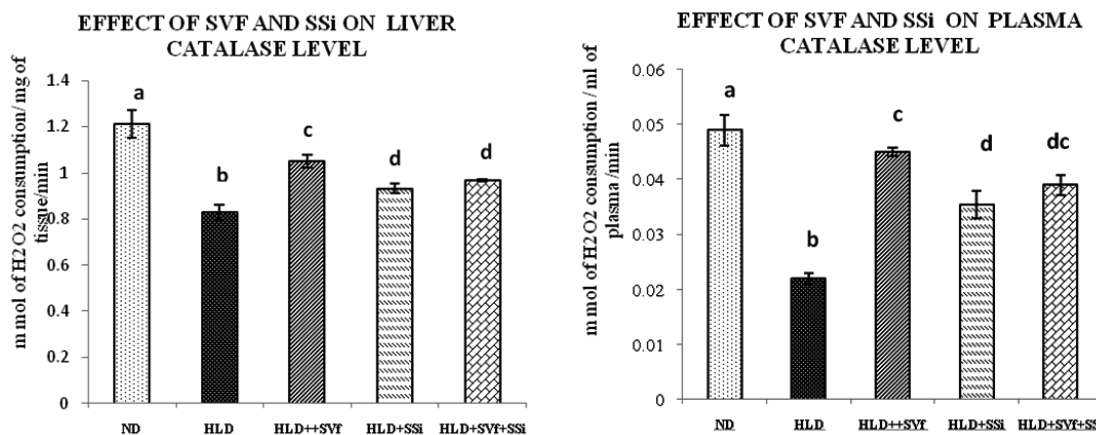


Fig. 1: Dietary Influence of SVf and SSi on liver and plasma Catalase level in high lipid fed male albino rats. Data are expressed as mean ± SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly ($p < 0.05$)

ND : Normal Diet; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba; HLD+SSi :High Lipid Diet + Sesame seeds; HLD+SVf+SSi: High Lipid Diet+ Seed of Vicia faba + Sesame seeds

and 21.62% in liver tissue by SVf+ SSi than the HLD. The elevated MDA concentration significantly ($p < 0.05$) countered by 10% SSi supplementation than the other two supplemented groups (Fig. 3).

Plasma GOT level significantly ($p < 0.05$) 30.86% increased and liver GOT level activity significantly ($p < 0.05$) 38.20% increased in HLD with respect to ND. SVf 10% supplementation significantly ($p < 0.05$) depleted 21.89% in plasma GOT and 17.36% depleted in liver GOT than the HLD. It is also observed that 35.34% decrement in plasma GOT level and 29.51% decrement in liver GOT level 10% SSi supplementation along with HLD in comparison to HLD. In supplementation with SVf+ SSi (5%+5%) significantly ($p < 0.05$) 32.21% diminution in plasma GOT and 38.62% diminution in liver GOT with comparison to HLD. Among the three dietary supplementation groups, 10% SSi supplemented group showed highest beneficial effect in plasma GOT level and combined supplementation (SVf + SSi) showed better positive influence in liver GOT level than the other two groups (Fig. 4).

It was demonstrated 22.95% elevation of plasma GPT and 26.70% elevation of liver GPT in HLD when compared with ND. In the same way, treated with

10% SVf significantly depleted 9.99% in plasma and 10.61% in liver tissue in respect to HLD. Similarly, 10% SSi fed rats presented depletion in 22.35% GPT in plasma and 22.89% GPT in liver than the HLD. The treatment with combined supplementation of SVf+ SSi (5%+5%) significantly decreased 20.48% plasma GPT and 20.38% liver GPT when compared to the HLD. From the above result, 10% SSi showed an expressive depletion of GPT level in plasma and liver than the other two supplemented groups (Fig. 5).

In this present study it was observed that HLD fed animals significantly increased 29.51% plasma IL-18 level compared with ND. After supplementation of 10% SVf and 10% SSi the level of IL-18 in plasma significantly 12.76% and 21.60% decreased as compared to HLD. In supplementation with SVf+ SSi (5% + 5%) significantly ($p < 0.05$) 29.25% diminution in plasma IL-18 with comparison to HLD. Among the three dietary supplementation groups, combined supplementation (SVf + SSi) showed high positive influence on plasma IL-18 concentration than the other two supplementation groups (Fig. 6).

The highest decreasing capacity of TG and TC was observed in HLD+SSi group, these values was nearest to the ND group probability due

Table 4: Effect of SVf and SSi on plasma and fecal lipid profile in high-fat fed male albino rats. Data are expressed as mean \pm SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly ($p < 0.05$)

| | ND | HLD | HLD+SVf | HLD+SSi | HLD+SVf+SSi |
|--------------------------------------|---------------------|--------------------|--------------------|---------------------|--------------------|
| Triglyceride (mg/dl) | 142.01 \pm 2.65a | 194.53 \pm 2.51b | 172.43 \pm 1.91c | 148.78 \pm 2.26 a | 159.68 \pm 2.96d |
| Total- Cholesterol (mg/dl) | 120.01 \pm 2.37 a | 202.96 \pm 1.02b | 174.26 \pm 1.96c | 136.4 \pm 3.17d | 147.83 \pm 3.30e |
| HDL-cholesterol (mg/dl) | 43.48 \pm 0.96 a | 32.75 \pm 0.89b | 36.06 \pm 0.51c | 41.76 \pm 1.08 a | 38.5 \pm 0.29d |
| LDL-Cholesterol (mg/dl) | 63.25 \pm 1.12 a | 103.41 \pm 1.10b | 83.41 \pm 0.91c | 62.8 \pm 0.97d | 70.93 \pm 0.96a |
| Fecal weight (g/day/rat) | 2.55 \pm 0.17 | 3.3 \pm 0.14 | 3.21 \pm 0.10 | 2.85 \pm 0.09 | 3.05 \pm 0.12 |
| Fecal total lipid(mg/g dried feces) | 58.2 \pm 0.84a | 96.64 \pm 0.95b | 55.5 \pm 0.99c | 78.24 \pm 1.05d | 66.23 \pm 0.81e |
| Fecal Cholesterol (mg/g dried feces) | 7.61 \pm 0.26a | 35.60 \pm 1.30b | 40.49 \pm 0.96c | 49.87 \pm 0.71d | 42.28 \pm 1.11ec |

ND: Normal Diet ;HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba ; HLD+SSi: High Lipid Diet + Sesame seeds; HLD+SVf+SSi : High Lipid Diet+ Seed of Vicia faba + Sesame seeds; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein.

to the presence of lignans, polyphenol, and flavonoid compounds, dietary fibers, PUFA, and lecithin in sesame.³⁷⁻³⁹ Many of the experimental

study confirmed that sesame slowdown the risk of cardiovascular disease.⁴⁰⁻⁴² Another evidence was high fecal cholesterol and high fecal lipid in

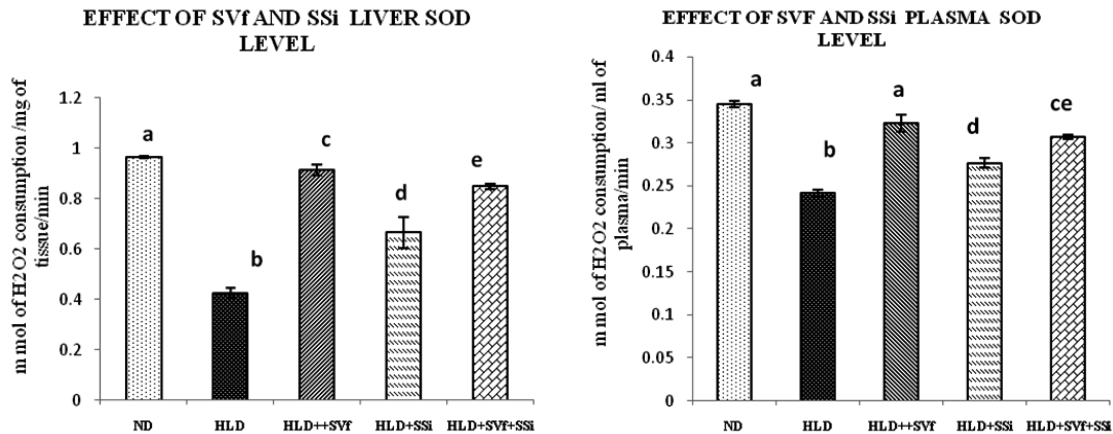


Fig. 2: The Role of dietary SVf and SSI on liver and plasma SOD level in high lipid fed male albino rats. Data are expressed as mean ± SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (p< 0.05)

ND : Normal Diet; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba; HLD+SSI :High Lipid Diet + Sesame seeds; HLD+SVf+SSI: High Lipid Diet+ Seed of Vicia faba + Sesame seeds.

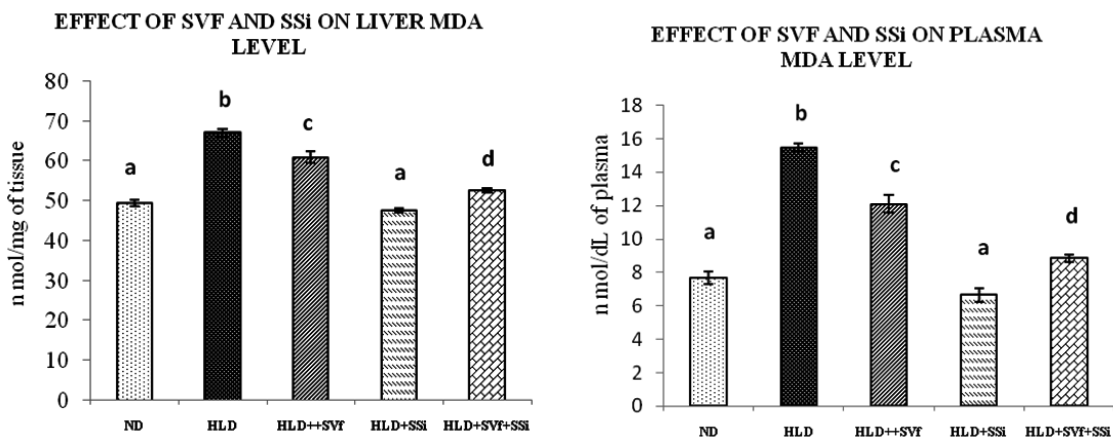


Fig. 3: Effect of dietary SVf and SSI on liver and plasma MDA level in high lipid fed male albino rats. Data are expressed as mean ± SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (p< 0.05).

ND : Normal Diet; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba; HLD+SSI :High Lipid Diet + Sesame seeds; HLD+SVf+SSI: High Lipid Diet+ Seed of Vicia faba + Sesame seeds.

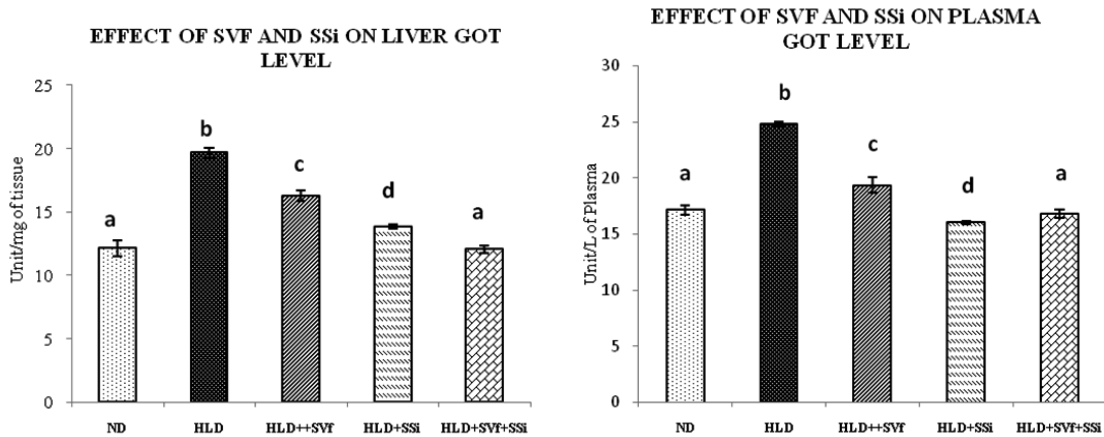


Fig. 4: Role of dietary SVf and Ssi on liver and plasma GOT level in high lipid fed male albino rats. Data are expressed as mean ± SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (p< 0.05).

ND : Normal Diet; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba; HLD+SSi :High Lipid Diet + Sesame seeds; HLD+SVf+SSi: High Lipid Diet+ Seed of Vicia faba + Sesame seeds.

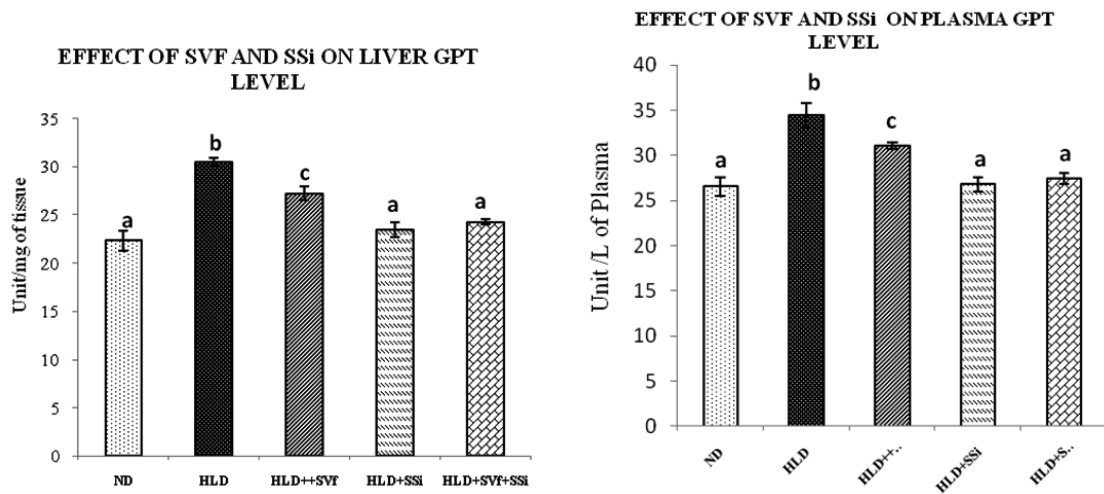


Fig. 5: Influence of dietary SVf and Ssi on liver and plasma GPT level in high lipid fed male albino rats. Data are expressed as mean ± SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly (p< 0.05).

ND : Normal Diet; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba; HLD+SSi :High Lipid Diet + Sesame seeds; HLD+SVf+SSi: High Lipid Diet+ Seed of Vicia faba + Sesame seeds.

HLD+SSi group than the other dietary supplemented groups. It has been suggested that TC and other lipids were removed via bile into fecal matter rather than transferred into the circulation indicated the fat-modified diet with HLD+SSi help to remove cholesterol via feces. On the other hand, LDL-C was decreased and HDL-C was increased in 3 supplemented groups when compared to HLD group. The highest positive effect was observed in HLD+SSi group result near to the ND group, test of hypothesis, high amount of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA). It was evidenced by increasing the level of fecal TC and decreased plasma TC in 3 dietary supplementary groups. Correlating low level of LDL-C, and HDL-C. Low level of plasma TC down regulates LDL-C receptors in extra-hepatic tissue which is one of the most important reasons to decreasing LDL-C and increasing HDL-C.³⁴

The present study also revealed that, the activity of CAT and SOD were significantly decreased ($p < 0.05$) in HLD group as compared to 3 supplemented

groups mainly due to high cholesterol, fat, energy diet while these parameters of in supplementary groups were increased due to low cholesterol and low plasma TG. Otherwise, MDA level in liver tissue in HLD group is high by elevated level of LDL-C, TG, and TC. The study showed that the lipid peroxidation level (MDA) is drastically high in HLD group. After treating with SVf, SSi and its composite manner the level of the MDA is significantly decreased ($p < 0.05$). In which the treatment HLD+SSi showed the good result than the other two treatment groups. SSi and SVf contain high level of omega-3 fatty acid about 10% more than omega-6 fatty acid which were analyzed in our laboratory (data were not shown in this paper). The study weakness was the fact that we did not analyze anti-inflammatory and pro-inflammatory markers, despite excellent compliance of over omega-3 and omega-6 fatty acids contents in SVf and SSi. Standard recommendation of ratio omega-3: omega-6 is 1:4 indicated to have beneficial effect. Omega-6 fatty acid like arachidonic acid and pro-inflammatory eicosanoids compound including prostaglandins - 2 (PG-2), thromboxane (TXA2), leukotrienes (LBT-4) and lipoxins in cell membrane. These pro-inflammatory eicosanoid increased lipid peroxidation and decreased the antioxidant enzyme level in the cell. Omega-3 fatty acid like eicosapentaenoic acid, docosahexaenoic acid and anti-inflammatory eicosanoids compounds including prostaglandins - 1 (PG-1), prostaglandins - 3 (PG-3) thromboxane (TXA2), leukotrienes (LBT-4) and lipoxins in cell membrane. These anti-inflammatory eicosanoids decreased the lipid peroxidation and increased the antioxidant enzyme level in the cell.⁴³ Evidence suggested that SVf and SSi contains a proportional amount of these fatty acids which might be one of the evidence to decrease lipid profile and lipid peroxidation. Earlier study have shown that there is a prominent relationship between hypercholesterolemia and some liver diseases due to high cholesterol deposits in the liver which can be oxidized by free radicals thereby causing hepatic injury.⁴⁴

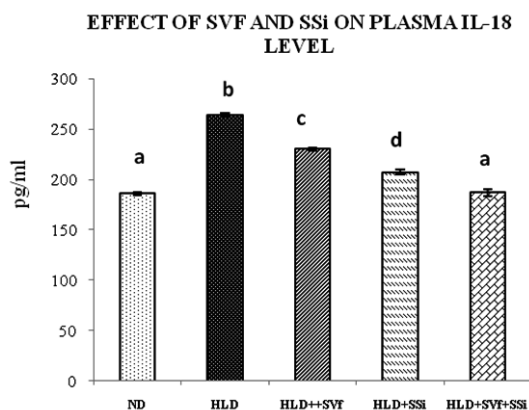


Fig. 6 Dietary effect SVf and SSi on plasma IL-18 level in high lipid fed male albino rats.

Data are expressed as mean \pm SE (n= 6). ANOVA followed by multiple two-tail t-test and data with different superscripts (a, b, c, d, e) in a specific vertical column differ from each other significantly ($p < 0.05$).

ND : Normal Diet; HLD: High Lipid Diet; HLD+SVf : High Lipid Diet + Seed of Vicia faba; HLD+SSi :High Lipid Diet + Sesame seeds; HLD+SVf+SSi: High Lipid Diet+ Seed of Vicia faba + Sesame seeds.

In this study, we observed that there were significant ($p < 0.05$) decrease in plasma and liver levels of GOT and GPT enzymes in HLD + SVf, HLD + SSi, HLD + SVf + SSi groups as compared to HLD fed animals. So, above three dietary supplementation had no general and metabolic toxic effect. This is

in agreement with many authors who reported the activities of ALT and AST high in high fat fed rats model which significantly normalization after the treatment with sesame meal and sesame oil.⁴⁵

HLD group rats fed diet with high cholesterol, high sucrose, coconut oil increases the plasma cholesterol level and which may cause dysregulated cholesterol metabolism in liver. Over burden of plasma cholesterol level activates monocytes and macrophages in artery, therefore monocytes and macrophages secrete the IL-18, evidenced by high concentration IL-18 in plasma in HLD group (Fig. 6).⁴⁶ Significant augmented plasma IL-18 level in supplementation group clearly support the plasma

cholesterol reducing the efficiency of SVf and SSi. Netea *et al.*, observed high plasma IL-18 positively correlated with plasma cholesterol level,⁴⁶ so our finding are strengthened by this report. Another evidence was proved by Bhat *et al.* increase oxidative stress and increase oxidation of LDL due to IL-18 administration in male mice.⁴⁷

Histopathological Analysis of Liver Tissue During Experimentation

Evaluate the effect of SVf, SSi, and SVf + SSi on liver histology in the dyslipidemic rats, liver tissues were examined under H and E staining. As shown in (Fig.7, Section B) after HLD fed, liver cells became extremely swelled and ballooned with severely

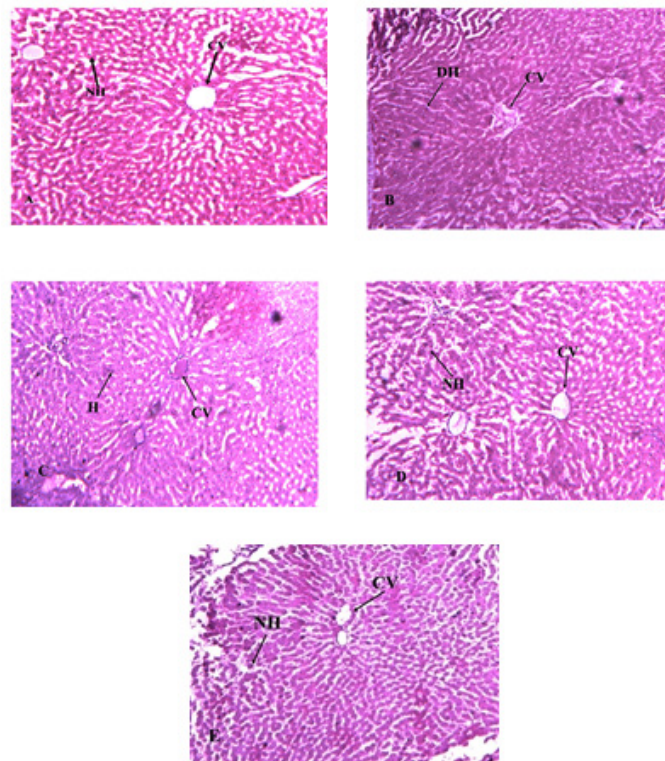


Fig. 7: Section A Showing normal histology of liver of ND group. Architecture shows radiated arranged hepatic cords around the central normal rat hepatic tissue (without fat). Architecture shows radiated arranged hepatic cords around the central venule. **Section B** Showing severe disorganization of rat liver cells after 60 days HLD fed rats, round the central vein (CV) are the hepatic cell cords. Intensive red coloring is the effect of high contents of fat inside hepatic cell. **Section C** showing for 60 days co-administered with SVf with HLD, showing less damaged hepatocytes (DH). **Section D** showing for 60 days HLD+SSi rats showing normal hepatocytes (NH) nearest to ND group. **Section E** showing HLD+SVf+SSi rats showing normal hepatocytes (NH) with a minimum disorganization (D).

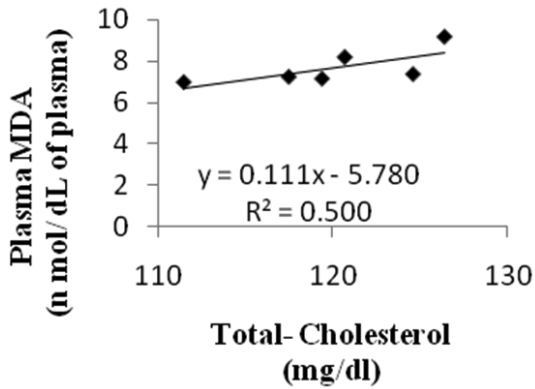


Fig. 8. A

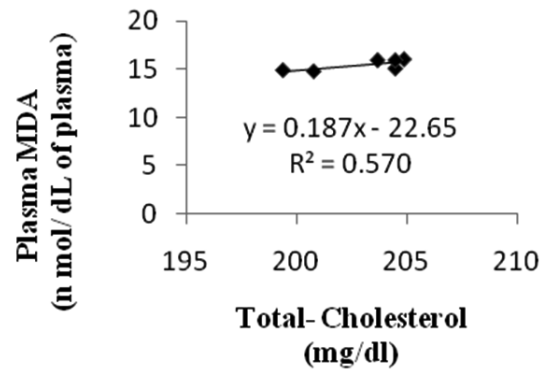


Fig. 8. B

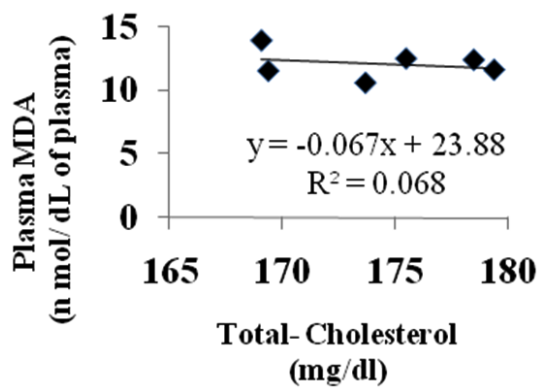


Fig. 8. C

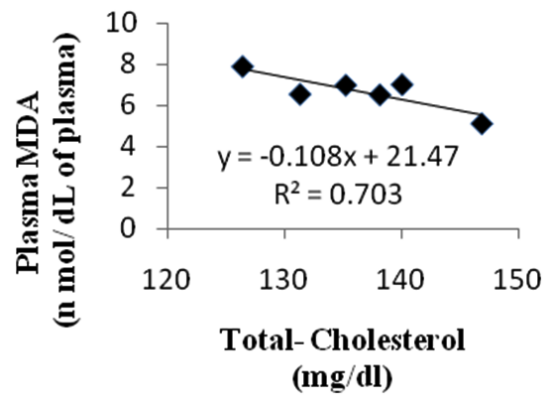


Fig. 8. D

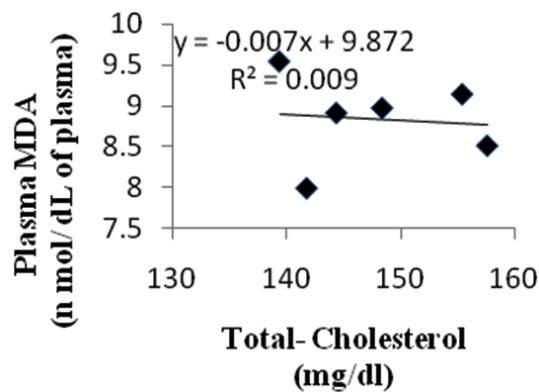


Fig. 8. E

Fig. 8: Correlation analysis was also represented graphically by scatter diagram. In all the scatter figures MDA values are taken on vertical axis and TC values are taken on horizontal axis. 8.A. represent the correlation between MDA and TC in ND group. 8.B. represents the correlation between MDA and TC in HLD group. 8.C. represent the correlation between MDA and TC in HLD+SVf group. 8.D. represent the significant correlation between MDA and TC in HLD+SSi group. 8.E. represent the correlation between MDA and TC in HLD+SVf+SSi group.

disorganized, around the central vein (CV) are the hepatic cell cords as compared with those from the ND fed rats (Fig.7, Section A). Intensive red coloring is the effect of high contents of fat inside hepatic cell, this result is proved by our earlier study where severe disorganization of liver cells and damaged hepatocytes and blood vessels were seen prominently due to the cause of high fat diet.²⁵ After 60 days of treatment of SVf with HLD liver pathological changes were partially improved as demonstrated by the decreased size fat cells in liver tissue and progress in blood vessel (Fig.7, Section C) but also co-administration of SSi with HLD was seen well-organized liver cells like normal CV and normal hepatocytes (NH) (Fig.7, Section D) as well as in ND group rats. This result was also supported by Azab *et al.*, where orally feeding sesame oil showed normal histological structure of liver tissue with organizes hepatocytes⁴⁸. In (Fig.7, Section E) there were the normal hepatocytes are seen as well as slightly affected ones with a small disorganization in the combination of SVf+ SSi when compared with SSi group rats.

Correlation Analysis: Lipid Peroxidation Versus Total Cholesterol

Correlation analysis was also represented graphically by scatter diagram. Plasma MDA values were taken on the vertical axis and plasma TC values were taken on the horizontal axis (Fig. 8). There was no significant ($p > 0.05$) correlation between plasma lipid peroxidation and increase in plasma TC in ND ($r = 0.708$) and HLD ($r = 0.756$) groups but high in lipid peroxidation showed high in total plasma TC in both high fat high cholesterol diet (Fig. 8, A,B). A weak downhill negative ($r = -0.261$) relation showed HLD + SVf supplementation and a very weak uphill positive ($r = +0.096$) relation showed HLD + SVf + SSi composite supplementation indicating the interfere of LPO by increasing the TC (Fig.8, C,E). There was weak relation between TC and LPO that resulted in the reduction of the level of LDL-C and higher the level of HDL-C. A strong downhill negative ($r = -0.839$) (Fig. 8, D) significant ($p < 0.05$) relation showed between TC and LPO in HLD+SSi supplementation group indicate increased the level of good cholesterol in plasma. A correlation result strongly hypothesized that SSi may play a good antidyslipidemic foods after deep investigation in molecular level in future.

Conclusion

High lipid diet induced dyslipidemia in rats (HLD) was proved to be evident by the increasing level of TC, TG, LDL-C, MDA, GOT, GPT, IL-18 and the decreasing level of HDL-C, SOD and CAT level than that of ND group. Only the sesame seeds (SSi) supplementation (10% of total foods) in diet showed significant decrease in TC, TG, LDL-C, MDA and significant increase in HDL-C level as compared to HLD, SVf and SVf + SSi groups. *Vicia Faba* (SVf) supplementation (10% of total foods) in the diet showed significantly increased SOD and Catalase than HLD, SSi and SVf + SSi groups. Combination of supplemented seed of *Vicia Faba* (SVf) (5% of total foods) + sesame seeds (SSi) (5% of total foods) showed decreased the level of IL-18, TC, TG, LDL-C, MDA concentration and increase the level of HDL-C, SOD, Catalase than the HLD group. Combination group showed the better result than the individual supplementation in respect of 5% SVf and 5% SSi (% arise from total food). Fifty percent reduction of seed of *Vicia Faba* and sesame seeds (w/w) combined supplementation results significant prevention of dyslipidemia which was an good synergistic effect. Supplementation quantity in combined treatment was reduced 50 % than individual supplementation. While recovery of dyslipidemia varied 10 to 20% in between individual and combined supplementation. So, finally we concluded that seed of *Vicia Faba* and sesame seeds combined supplementation might be accepted and potential against dyslipidemia and further research in future.

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Conflict Interest

I declare that there is no conflict of interest regarding the publication of this paper which titled "Supplementation of seed dust of *Vicia Faba* and sesame ameliorates high lipid diet-induced dyslipidemia in rats".

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