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Evaluation of attenuative potential of flaxseeds in high-calorie, high-fat, and fructose-induced metabolic syndrome indicators in Wistar rats without dietary reversal

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ABSTRACT

The study investigates the effects of flaxseed extract on high-calorie, high-fat, and fructose-fed metabolic syndrome parameters in male Wistar rats without diet reversal, encouraged by increasing western diet-induced obesity-associated metabolic syndrome incidence and anti-hyperlipidemic effects of flaxseeds. Fifty-six male Wistar rats were divided into seven groups (n = 8/group); the first group (normal controls) received a normal diet, and the second group (flaxseed controls) received a normal diet and flaxseed extract (1.0 g/kg body weight/day) orally for 16 weeks. The third group (metabolic syndrome controls) received a high-calorie, high-fat diet and fructose for 16 weeks, with induction observed at 8 weeks. The fourth and fifth groups received a high-calorie, high-fat diet and fructose for 16 weeks with flaxseed extract initiated after 8 weeks with doses of 0.5 and 1.0 g/kg body weight/day (post-exposure groups). The sixth and seventh groups received a high-calorie, high-fat diet and fructose with flaxseed extract doses of 0.5 and 1.0 g/kg body weight/day for 16 weeks (co-exposure groups). High-dose flaxseed extract reduces body weight, blood glucose, cholesterol, triglycerides (TAGs), uric acid, and total oxidant status while increasing high-density lipoprotein. Liver histopathology indicates that high-dose flaxseed extract co-exposure protects against fructose-induced hepatic steatosis. Thus, without dietary alterations, flaxseeds lower blood sugar, cholesterol, TAG and improve hepatic steatosis, attenuating metabolic syndrome markers induced by high-calorie, high-fat, and fructose diets.

INTRODUCTION

Metabolic syndrome is an assemblage of metabolic and systemic alterations, including obesity, hyperglycemia, hyperinsulinemia (due to insulin resistance), dyslipidemia, and hypertension [elevated systolic blood pressure (SBP) and diastolic blood pressure (DBP)] caused by nutrient overload, storage, and reduced energy expenditure. According to the NCEP ATP III guidelines, metabolic syndrome diagnosis requires the presence of at least three of the following characteristics: abdominal obesity, fasting sugars ≥ 110 mg/dl, dyslipidemia [increased triglycerides (TAGs), total cholesterol, decreased high-density lipoprotein (HDL) cholesterol], elevated SBP, or DBP. The current body of the literature suggests a global landscape change in the incidence rates of metabolic syndrome wherein 23.7% of type 1 diabetic patients have metabolic syndrome [1] and an alarming increased incidence of metabolic syndrome among children being 2.8% and 4.8% for adolescents, with higher incidence rates observed in low-income countries [2]. Emerging studies indicate a higher incidence rate among

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Asian Indians, with the majority of the affected individuals commonly exhibiting a reduction in HDL-cholesterol levels [3] with some studies indicating incidence rates in India to be 30% [4] with obesity or overweight individuals presenting a higher risk of developing metabolic syndrome [5].

One of the significant attributable risks for developing metabolic syndrome is overconsumption or nutrient excess conditions caused by increased consumption of energy-dense, high-fat, or high-fructose/sucrose-containing palatable foods and concomitant reduction in energy expenditure due to a sedentary lifestyle. One of the earliest changes observed due to the consumption of a high-calorie or high-fat diet is the phenotype of the gut microbial population, which effectively leads to reduced production of beneficial short-chain fatty acids (SCFAs) such as butyrate and propionate, altering the response of gut immune cells such as innate lymphoid cells, T- and B-lymphocytes, increased permeability of the gut epithelial barrier lining [6] increasing the transmigration of lipopolysaccharides from the gut lumen into systemic circulation causing the activation of the immune system. The overall consumption of fructose or sucrose-containing dietary items has increased in the last 10 years [7], which has had drastic effects on the overall metabolic profile since fructose consumption induces *de novo* lipogenesis in the liver. Fructose metabolism also aids in the production of advanced glycation end products such as methylglyoxal and N-(Carboxymethyl) lysine which have independent or cumulative effects on metabolic alterations, thus predisposing individuals to increased risk of developing obesity or metabolic syndrome [8]. The excess dietary carbohydrates and lipids absorbed are converted into fats and stored in adipose tissues, leading to hypertrophy and hyperplasia of adipose tissue, which alters the profile of multiple adipokines released from the adipose tissues, severely impacting the metabolic homeostasis, leading to a functional reduction in insulin action leading to insulin resistance further accentuated by activation of the immune system and release of pro-inflammatory cytokines. One of the significant adipokines altered in metabolic syndrome is adiponectin [9], which is reduced with adipose tissue expansion, causing an overall reduction in energy expenditure. Fructose consumption stimulates hepatic lipogenesis, producing TAGs which are transported to the adipose tissue via very low-density lipoproteins (VLDLs) [10,11]. The constant storage of lipids in adipocytes coupled with dysfunctional mobilization leads to saturation of both subcutaneous and visceral adipocytes, which initiates the spill-over mechanism leading to ectopic deposition of lipids in other tissue such as liver [12] and increasing the levels of circulating free fatty acids (FFAs), TAGs, cholesterol and associated lipoproteins such as low-density lipoprotein (LDL), VLDL but decreasing HDL. The over-expanding adipocytes start suffering from hypoxia, which initiates the hypoxic response leading to adipose tissue extracellular matrix (ECM) remodeling and anaerobic metabolism of glucose-inducing lactate production that, in turn, induces greater lipid uptake metabolic reprogramming of adipose tissue immune cells [13] such as macrophages toward the pro-inflammatory M1 profile further inducing the production of pro-inflammatory cytokines and a state of low-grade metainflammation. The elevated levels of circulating FFAs also induce the production of other lipid derivatives such as ceramides, which, along with FFAs and increased metabolic load, induce endoplasmic reticulum (ER) and mitochondrial stress in cells [14]. The existing pro-inflammatory cytokines, ceramide, FFA-induced ER, and mitochondrial stress inhibit the insulin signaling downstream pathway, which reduces insulin action, causing hyperglycemia and compensatory hyperinsulinemia [15]. The relative inefficiency of insulin and fructose-metabolism byproduct uric acid inhibits endothelial nitric oxide (NO) synthesis, which reduces vasodilation and high-fat, high-calorie-induced angiotensin II activation in adipocytes increasing the blood pressure (SBP and DBP) [16]. The increased load of lipids and pro-inflammatory immune cell activation stimulates the hepatocytes' metabolic profile, reducing autophagy and increasing the activation of hepatic stellate cells and Kupffer cells, inducing hepatic ECM modification and causing steatohepatitis [17,18].

Flaxseed (*Linum usitatissimum*), the oldest cultivated crop, produces oil, provides food, and has appreciable amounts of soluble and insoluble fiber. It is a good source of the omega-3 fatty acid, linolenic acid [19], linoleic, oleic, palmitic, and stearic acids; tocopherols; sterols; sterol esters, phospholipids, waxes, FFAs, and carotenoids. Flaxseeds also contain cyanogenic glycosides, namely mono-glycosides such as linamarin and lotaustralin, and di-glycosides such as linustatin and neolinustatin and secoisolariciresinol diglucoside (SDG) [19], with SDG characterized as a flaxseed lignan polymer composed of phenylpropanoids and hydroxymethyl glutaric acid [20–24]. Some studies have also shown that including flaxseeds in the diet significantly helped reduce total plasma cholesterol, TAGs, and LDL-cholesterol. Still, the results are inconclusive since some studies have reported no effect on HDL-cholesterol levels or lipid levels in animal studies [25–29]. Studies also indicate that SDG may reduce DBP [30], and clinical studies involving peripheral arterial disease patients with high blood pressure reported that consuming flaxseeds for more than 3 months lowered blood pressure [31]. SDG also exhibits an inhibitory effect on lipogenic genes with concomitant stimulation of lipolytic genes [32]. Some studies have also indicated that flaxseed lignans regulate adipogenesis via modulation of peroxisome proliferator-activated receptor-gamma expression, which may also enhance GLUT-4 translocation in insulin-sensitive tissue and upregulation of adiponectin expression, which may help reduce insulin resistance [33]. Studies in animal models also indicate that flaxseed lignan (SDG) improves glycemic control in animal models (but not whole flaxseeds) [34] and ameliorates central obesity and inflammation by altering the gut microbiome in mouse models [35]. Flaxseed derived ω-3 fatty acids can promote the abundance of *Bifidobacteria* [36,37], flaxseed consumption increases *Akkermansia muciniphila* [22], *Lactobacilli* (*Lactobacillus gasseri* and *reuteri*) impairs lipid absorption [39–41], and *Bifidobacterium*, *Clostridium*, and *Bacteroides* [23] reduce this Firmicutes/Bacteroides ratio by promoting the comparative abundance of firmicutes [38,42,43]. Flaxseed consumption improves glycemic control in prediabetic obese men and women [24].

One of the significant lacunae identified from studies with flaxseed administration is the lack of induction or presence of metabolic syndrome parameters focusing on inspecting

the effects of flaxseeds in hyperlipidemic or T2DM animals/humans with conflicting results [31,33,34,44–60]. In one study with metabolic syndrome induction in rodents [20], the dietary pattern was altered from a high-fat, high-calorie diet to normal diet, with studies demonstrating that dietary reversal from high-fat to normal diet by itself can induce metabolic alteration in rodents [61–63] and may even impact the epigenome thusaltering the overall energy expenditure via the hypothalamic network [64]. Therefore, our work focuses on identifying the metabolic alterations induced by different doses of flaxseed extract supplementation in the event of co-exposure or post-induction exposure to a high-fat, high-calorie diet and fructose-induced metabolic syndrome parameters in male Wistar rats without diet reversal.

MATERIALS AND METHODS

Diets

Normal Lab Diet 5L79 (Energy- 3.15 Kcal/g (13.18 KJ/g) obtained from Hylasco Biomedicals, Hyderabad, India.

High-calorie, high-fat diet (HCHF): 3.725 Kcal/g (15.585 KJ/g) was made by mixing Lab Diet 5L79 with cholesterol Sisco Research Laboratories Pvt. Ltd. (SRL), sucrose Loba Chemie (LOBA), sodium cholate SRL, casein (Vitamin-free casein, Himedia), and anhydrous milk fat in the compositional ratio of 48.5 % Lab Diet 5L79, 10% casein (vitamin-free casein), 30% sucrose, 10% anhydrous milk fat, 1% cholesterol, and 0.5% sodium cholate.

Fructose LOBA 60% (w/v): 2.4 Kcal/ml (10.04 KJ/ml).

Preparation of hydroethanolic extract of flaxseeds

Commonly (commercially) available flaxseeds were purchased, sun-dried, and microwave-dry roasted for about 15 minutes at 40°C followed by grinding to a fine powder. It was milled and passed through a sieve (mesh 300) and stored in a desiccator at 4°C. One hundred grams of powdered seeds were used for extraction using a warm maceration method with 80% (v/v) ethanol for 72 hours in the dark, with extract separation done by filtration every 24 hours, followed by fresh solvent replacement. The filtrate was concentrated under the rotary evaporator and dried in the oven at 40°C for 48 hours to obtain a dark-brown powder, which was stored (extraction ratio was 10:1) in a desiccator at 4°C until use [65]. The dark-brown powder was dissolved in distilled water with vortex mixing and left undisturbed for 24 hours with filtration to remove any suspended particles to produce a stock solution (1.0 g/ml).

Animals used

Fifty-six male Wistar rats, 3–4 months old, weighing 250 ± 25 g, utilized for the study were obtained from the Central Animal Research Facility (CARF), MAHE, Manipal, India. The rats were acclimatized for one week before the initiation of the study and maintained at a room temperature of 24°C ± 2°C, with a standard 12-hour light/dark cycle with free access to food and water throughout the entire study. Rats were individually housed, with all efforts made to minimize the suffering of rats.

Experimental design

Fifty-six male Wistar rats were divided into seven groups of eight animals (n = 8/group) with efforts to maintain constant body weight ranges across groups.

Group 1: Normal controls (NCs): Fed with a normal Lab Diet 5L79 for 16 weeks.

Group 2: Metabolic syndrome controls (MS): Fed with a high-calorie, high-fat diet with an additional administration of 1 ml/kg of 60% fructose solution (w/v) in distilled water daily via oral gavage for 16 weeks, with metabolic syndrome symptoms induction seen at 8 weeks.

Group 3: Low-dose post-exposure group (MS+F-G1): Put on a high-calorie, high-fat diet and 1 ml/kg of 60% fructose solution for 8 weeks, followed by the introduction of flaxseed extract at the dose of 0.5 g/kg body weight via oral gavage for the next 8 weeks with the continuation of high-calorie high-fat diet and 60% fructose with sufficient time intervals between doses.

Group 4: High-dose post-exposure group (MS+F-G2): Fed with a high-calorie, high-fat diet and 1 ml/kg of 60% fructose solution for 8 weeks, followed by the introduction of flaxseed extract at the dose of 1.0 g/kg body weight via oral gavage for the next 8 weeks with the continuation of high-calorie high-fat diet and 60% fructose with sufficient time intervals between doses.

Group 5: Low-dose co-exposure group (F+MS-G1): Put on a high-calorie, high-fat diet and 1 ml/kg of 60% fructose solution with flaxseed extract at the dose of 0.5 g/kg body weight via oral gavage for the entire 16 weeks, with sufficient time intervals between oral doses.

Group 6: High-dose co-exposure group (F+MS-G2): Administered a high-calorie, high-fat diet and 1 ml/kg of 60% fructose solution with flaxseed extract at the dose of 1.0 g/kg body weight via oral gavage for the entire 16 weeks with an adequate time gap between the oral gavages.

Group 7: Flaxseed control (FLXC): Fed with a normal Lab Diet 5L79 and flaxseed extract at a 1.0 g/kg body weight dose via oral gavage for 16 weeks.

The male rats were weighed weekly with measurement of abdominal circumference (AC) (measured with a non-expandable tape immediately anterior to the forefoot) and nose-anal length. For an anthropometric assessment, body mass index (BMI) was calculated using the formula BMI = body weight (g)/length^2 (cm), body fat index (Lee index [66–68]) calculated using the formula (cube root of body weight (g)/nose-to-anus length (cm)). The flaxseed extract dosage of 0.5 g/kg body weight (low-dose) was decided based on its protective effects seen from a colitis model in rats [69]. The high-dose (1.0 g/kg body weight) was double the low dose (one-fifth of the limit dose of 5,000 mg/kg body weight) and did not have any acute toxicity effects, as indicated by other studies [70]. The total experimental duration was 16 weeks, with blood collection at baseline, 8, and 16 weeks in fluoridated tubes and non-fasting clotted blood samples. The choice of experimental duration was based on previous studies indicating hyperinsulinemia due to a high-fat and/or high-fructose diet will be induced by 8 weeks [71], which was seen in our study as indicated by the development of hyperglycemia, dyslipidemia, abdominal

obesity, and BMI elevations. The overall unhealthy effects with structural and physiological abnormalities in organs such as the liver, pancreas, and adipose tissue may be observed by week 16, thus prompting the total experimental duration [71].

The animals were fasted (overnight) for 12 hours before blood collection, at baseline, after the completion of 8 weeks (on the first day of the ninth week), and at the end of 16 weeks (on the first day of the 17th week). Serum and plasma were collected to estimate the fasting glucose and lipid profile using standard biochemical kits (Agappe Diagnostics). Fasting insulin and adiponectin were also measured using standard ELISA kits (Elabscience Biotechnology Ltd). Serum uric acid levels (Agappe Diagnostic kits), NO metabolites [72,73], and total oxidant status (TOS) [74] were also measured. After 16 weeks, fasting plasma and serum were collected, followed by euthanasia and dissection of Wistar rats. The weight of fat pads (abdominal, epididymal, and subcutaneous) and liver was recorded. The visceral adiposity index (VAI) was calculated from the total weight of the adipose fat pads using the formula (total body fat/final body weight) × 100 [68,75,76], where total body fat was the sum of epididymal, retroperitoneal, and visceral fat.

Liver tissue was collected, weighed, and kept for histopathological assessment in neutral buffered formalin followed by treatment with sequential changes in ethanol solutions of increasing concentration followed by hematoxylin and eosin staining using standard protocols, after which non-alcoholic steatohepatitis (NASH) histopathological grading was done [77].

Statistical analysis

All the statistical tests were performed using Jamovi software version 2.3. Weekly changes in body weight and AC for 16 weeks were analyzed using repeated measure ANOVA after meeting the normality assumption using the Shapiro–Wilk normality assessment. BMI, Lee's Fat Index, and biochemical parameters at baseline, 8 weeks, and 16 weeks were evaluated for Shapiro–Wilk normality. Study parameters that agreed with normality assumptions were analyzed using parametric one-way ANOVA with Tukey's post hoc tests with statistical significance accepted at $p \leq 0.05$ and results reported as mean ± SD. For parameters violating Shapiro–Wilk normality estimations, Kruskal–Wallis non-parametric tests were performed with $p \leq 0.05$ considered statistically significant and results reported as median Inter-quartile range (IQR).

RESULTS

Anthropometric measurements

In rats receiving a high-calorie, high-fat diet and fructose, the body weight increased significantly compared to normal and FLXCs. Still, groups where flaxseed extract was introduced from day one had significantly lower body weights than groups without flaxseed extract (Table 1). Body weight and abdominal variations across groups in 16 weeks are indicated in Figures 1 and 2. An anthropometric indicator, such as BMI analysis, using Fisher's one-way ANOVA at 8 weeks, shows a statistically significant difference between groups ($F_{[6,49]} = 16.516$, $p < 0.001$), with post hoc comparisons using Tukey's HSD (Honestly Significant Difference) revealing that groups receiving only HCHF, and fructose showed a significant increase in BMI when compared to NCs. In contrast, the groups that received flaxseed extract, HCHF, and fructose demonstrated no difference in BMI between them and NCs. At the end of 16 weeks, both flaxseeds extract post-exposure (8 weeks onwards), and co-exposure showed a lower BMI as compared to metabolic syndrome controls ($F_{[6, 49]} = 47.339$, $p < 0.001$). Still, co-exposure groups exhibited a lower BMI than post-exposure groups (Fig. 3).

The body fat index at 8 weeks was significantly different among groups demonstrated by Fisher one-way ANOVA ($F_{[6, 49]} = 36.761$, $p < 0.001$). Tukey's post hoc analysis revealed that flaxseed extracts co-exposure groups showed a lower fat index than non-exposure groups when administered an HCHF and fructose dietary regime (Fig. 4). At 16 weeks, Fisher's one-way ANOVA ($F_{[6,49]} = 78.769$, $p < 0.001$) test statistics indicated flaxseed extract lowered body fat index when compared to metabolic syndrome controls and Tukey's post hoc comparison co-exposure groups showed a lowered body fat index as compared to post-exposure groups (Fig. 4).

VAI analysis at 16 weeks, using a Kruskal–Wallis test, indicated a significant difference between groups [$\chi^2(6) = 29.0$, $p < 0.001$, $\epsilon^2 = 0.528$], and pairwise comparison reveals

Table 1. Body weight, AC at baseline, 8 and 16 weeks.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Body weight: 0 days (in grams)	253 ± 5.65	259 ± 13.48	261 ± 6.09	255 ± 10.95	262 ± 6.02	265 ± 6.99	264 ± 7.92
Body weight: 8 weeks (in grams)	318 ± 8.90	428 ± 19.46 a***	408 ± 17.03 a***	399 ± 31.57 a***	342 ± 17.82 b***, c***	336 ± 15.76 b***, c***	329 ± 14.02
Body weight: 16 weeks (in grams)	340 ± 13.04	511 ± 18.39 a***	438 ± 17.19 a***	422 ± 32.50 a***	361 ± 23.10 b***, c***	354 ± 14.35 b***, c***	323 ± 16.82
AC: 0 days (in cm)	15.0 ± 0.61	15.5 ± 0.43	15.7 ± 0.58	15.0 ± 0.61	15.1± 0.72	15.3 ± 0.71	15.2 ± 0.89
AC: 8 weeks (in cm)	15.8 ± 0.49	18.0 ± 0.25 a***	18.7 ± 0.55 a***	18.5 ± 0.72 a***	15.7 ± 0.54 b***, c***	16.0 ± 0.89 b***, c***	15.6 ± 0.93
AC: 16 weeks (in cm)	16.0 ± 0.52	20.2 ± 0.49 a***	17.1 ± 0.61 a*	17.1 ± 0.74 a*	15.1 ± 0.49 b***, c***	15.5 ± 0.89 b***, c***	14.9 ± 0.76

Data are presented as mean ± SD. Differing superscript letters indicate differences between comparison groups where a—compared to NC, b—compared to MS controls, c—compared to groups 3 and 4, * indicates $p \leq 0.05$, and *** indicates $p \leq 0.001$.

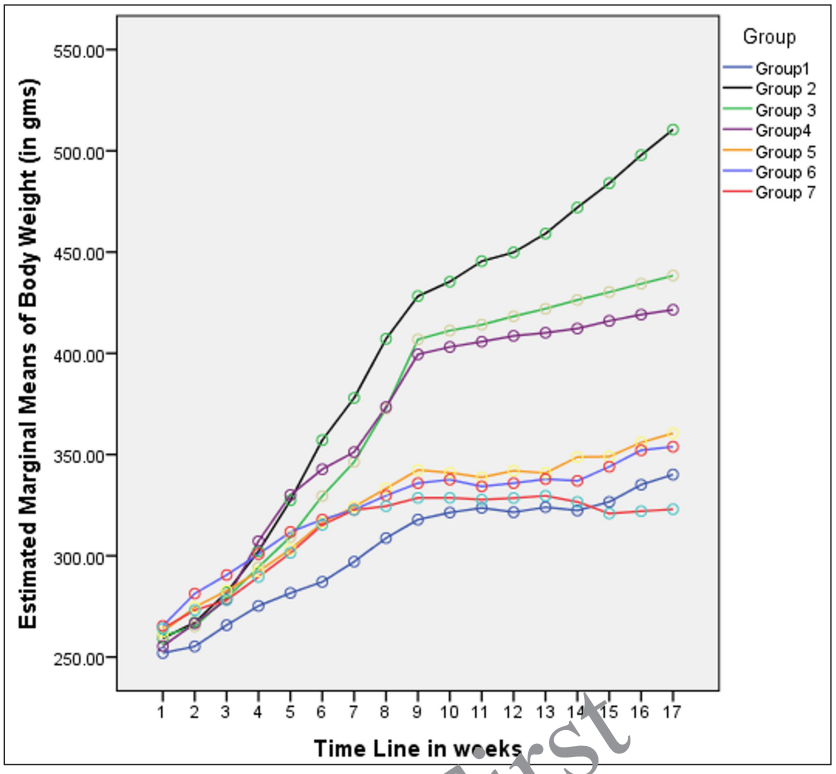

Figure 1. Variations in body weight (in grams) among different groups in 16 weeks.

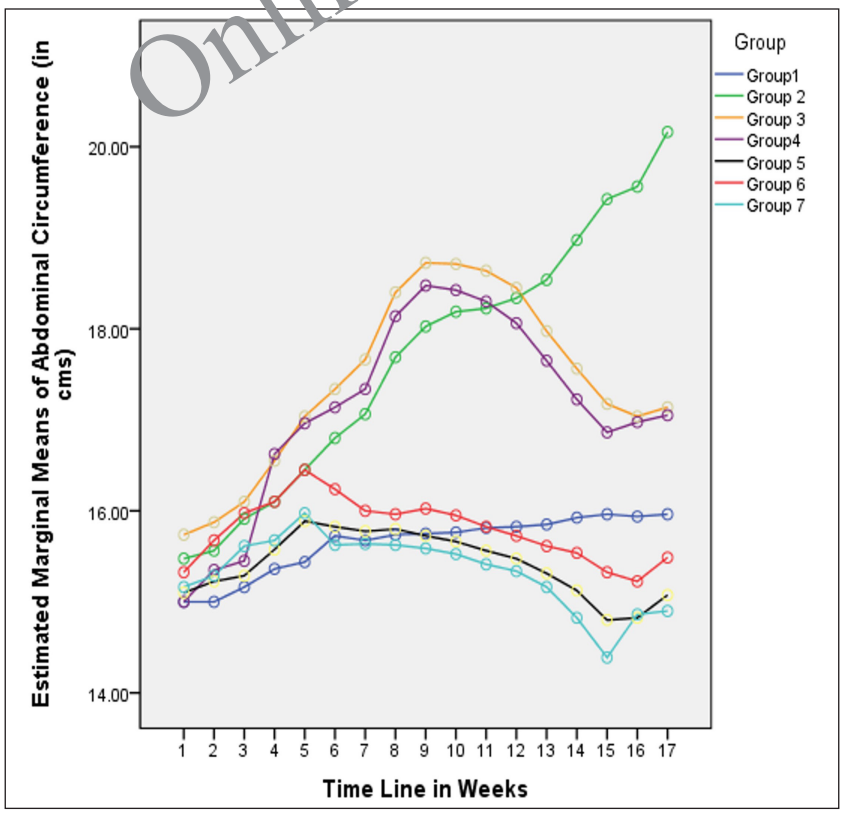

Figure 2. Variations in AC (in cm) among different groups in 16 weeks.

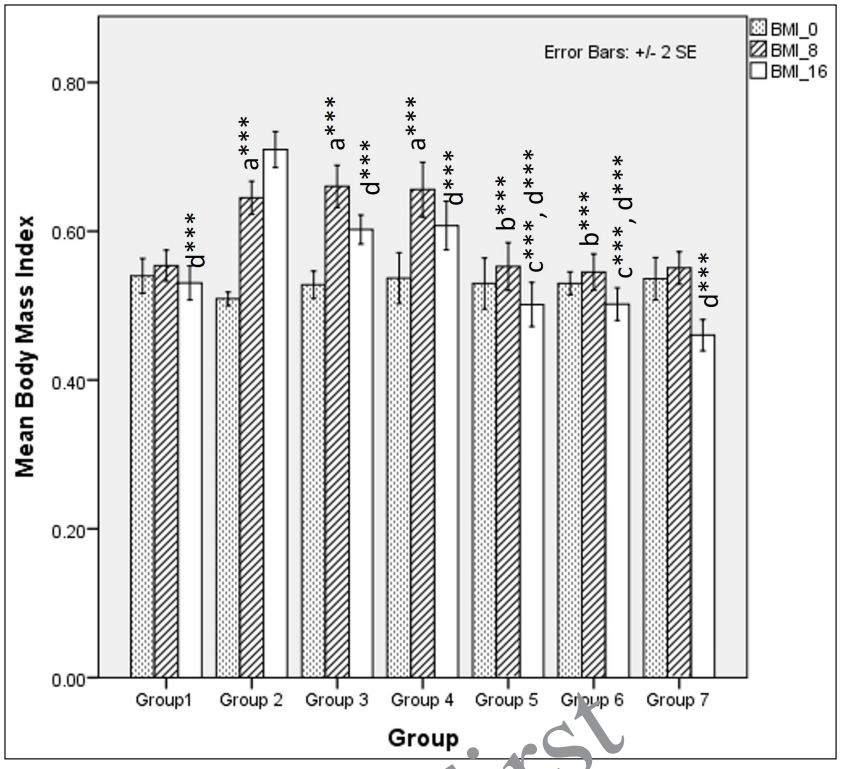

Figure 3. Comparison of BMI at 0 days. 8 and 16 weeks. a represents comparison to group 1, b represents comparison to group 3,4 and 5, c represents comparison to groups 3 and 4, d represents comparison to group 2 (Fisher's one-way ANOVA with Tukey's post hoc analysis) (*** p-value < 0.001, ** p <0.01, * p <0.05).

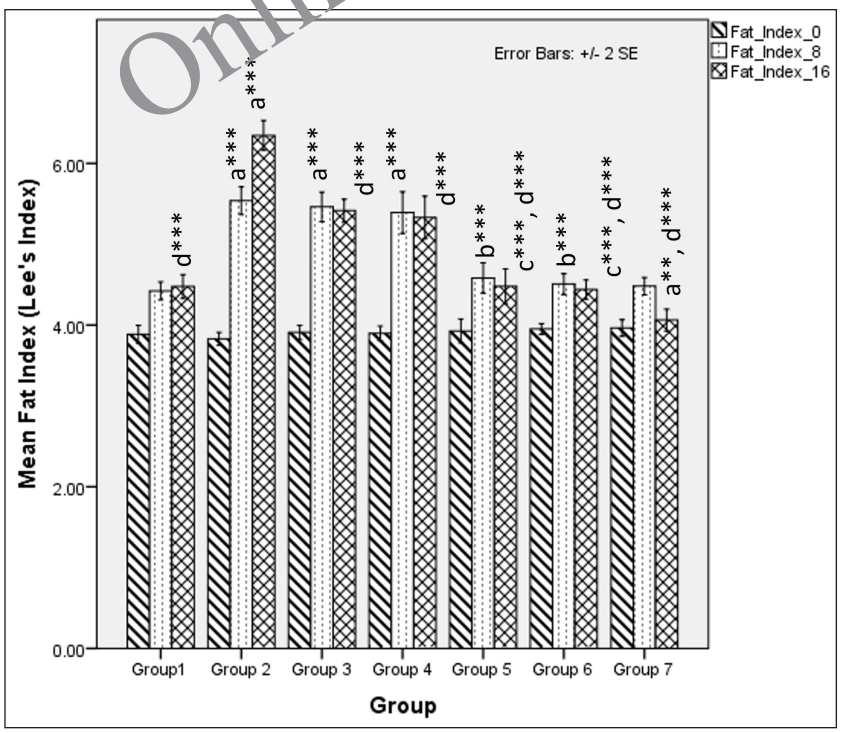

Figure 4. Comparison of body fat index (Lee's index) at 0 days. 8 and 16 weeks. a represents comparison to group 1, b represents comparison to groups 3, 4 and 5, c represents comparison to groups 3 and 4, d represents comparison to group 2 (Fisher's one-way ANOVA with Tukey's post hoc analysis) (*** p-value < 0.001, ** p <0.01, * p <0.05).

a significant difference in visceral adiposity between metabolic syndrome controls versus NCs, high-dose flaxseed extract post-exposure, co-exposure, and control groups (Fig. 5).

At 16 weeks, subcutaneous fat accumulation also indicated a significantly lower fat content in flaxseed receiving groups when compared to metabolic syndrome controls using Kruskal–Wallis test statistics [χ^2 (6) = 27.5, $p < 0.001$, ϵ^2 = 0.500], with pairwise comparison indicating a statistically significant difference between metabolic syndrome controls and high and low-dose flaxseed extract post-exposure groups and high-dose co-exposure groups (Fig. 6).

Glucoregulatory estimations

Fasting plasma glucose at 8 weeks exhibited a statistically significant difference when compared between HCHF and fructose-consuming groups with and without flaxseed extract co-administration via one-way ANOVA (Fisher's) F [6,49] = 23.0, $p < 0.001$. At 16 weeks, there was a difference in fasting plasma glucose levels among groups one-way ANOVA (Welch's) F [6,21.5] = 22.7, $p < 0.001$ all the groups receiving HCHF and fructose had statistically significant elevated fasting blood glucose as compared to NCs and flaxseed extract administration both post and co-exposure exhibited a lower fasting plasma glucose when compared to metabolic syndrome controls (Welch's one-way ANOVA with Games–Howell post hoc) (Table 2) which was statistically non-significant, but it may be clinically significant since the mainstay of any hyperglycemic conditions treatment actively targets reduction of plasma sugars.

Homeostatic assessment of insulin resistance (HOMA-IR) at different intervals also depicts a similar pattern as plasma sugars with initial increases in insulin resistance with HCHF and fructose consumption. Flaxseed extract co-administration reduces insulin resistance (Kruskal–Wallis χ^2 (6) = 49.7, $p < 0.001$, ϵ^2 = 0.903) with pairwise comparison revealing that values of HOMA-IR in flaxseed extract receiving groups were significantly lower than high-calorie high-fat and fructose consuming groups. At the end of 16 weeks, HOMA-IR values significantly differ among different groups (Kruskal–Wallis χ^2 (6) = 45.2, $p < 0.001$, $\epsilon^2 = 0.821$), with significant reduction observed when compared between metabolic syndrome controls and flaxseed extract receiving groups (Table 3, Figs. 7 and 8).

Fasting serum lipids

In the first 8 weeks, the ingestion of HCHF and fructose causes a significant difference in serum fasting total cholesterol levels between groups (one-way ANOVA Welch's F [6,20.6] = 155.7, $p < 0.001$) where Games–Howell post hoc analysis revealed that all groups receiving HCHF and fructose had higher total cholesterol compared to NCs ($p < 0.001$). Still, flaxseed extract exposure groups showed significantly lower serum cholesterol than non-exposure groups ($p < 0.05$). At 16 weeks, total cholesterol levels differed distinctly among groups one-way ANOVA Welch's F [6,20.4] = 20.9, $p < 0.001$) with Games–Howell post hoc analysis indicating a reduction in

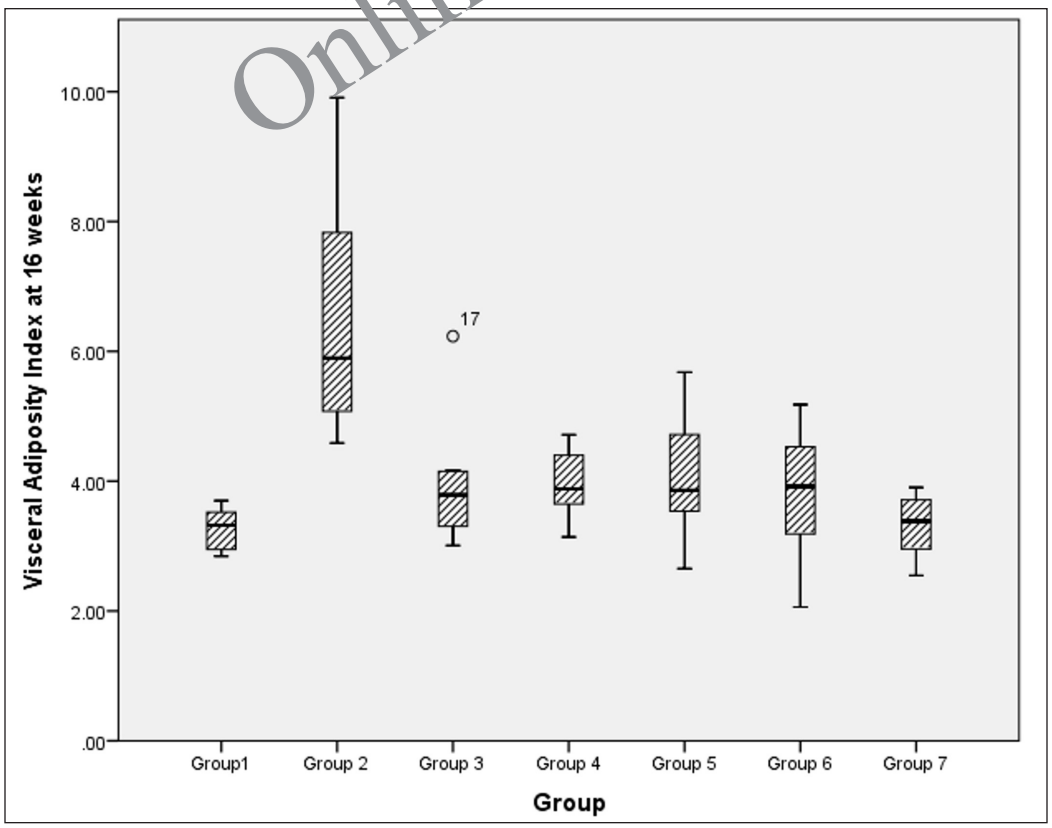

Figure 5. Visceral adiposity index at 16 weeks among different groups.

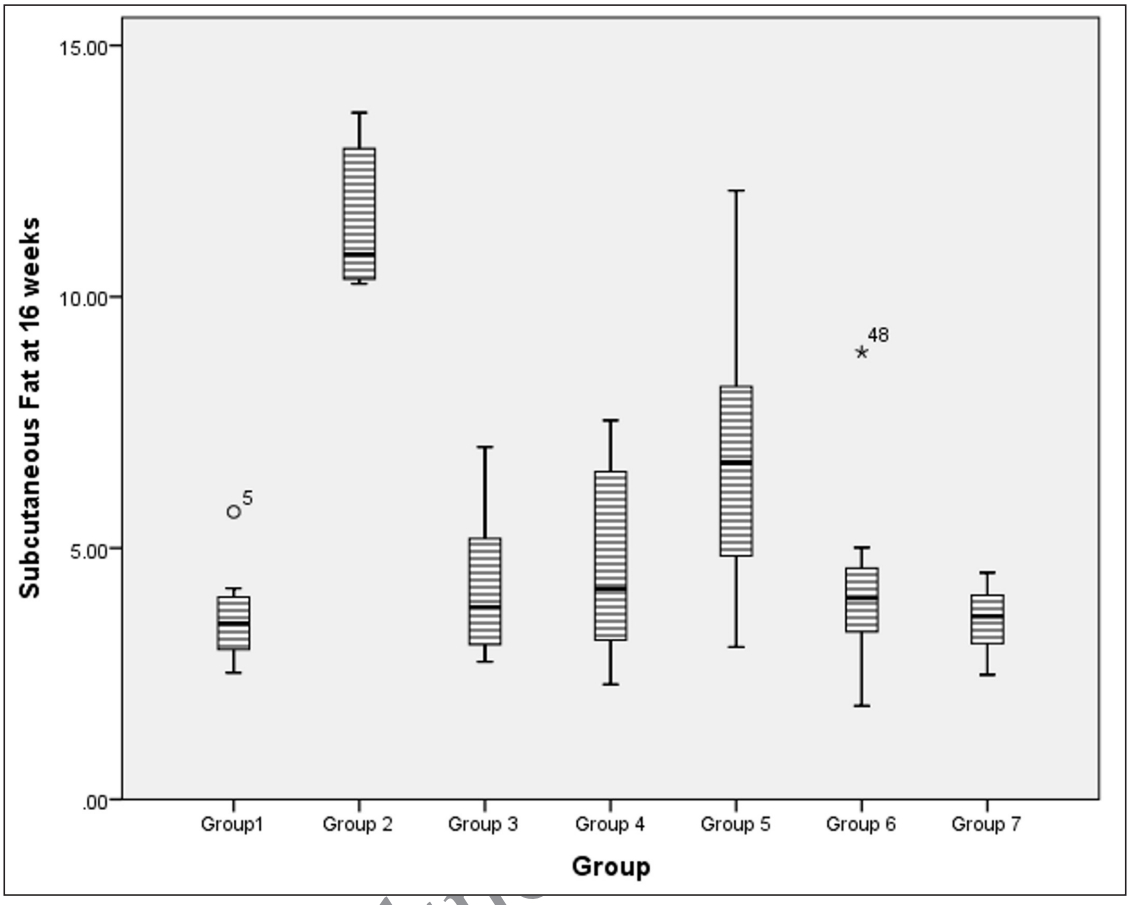

Figure 6. Amount of subcutaneous fat accumulation (in grams) at 16 weeks in different groups.

Table 2. Comparisons of fasting blood glucose and insulin levels between groups at different time points.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Fasting glucose: 0 days (in mg/dl)	80.6 ± 10.36	84.1 ± 12.22	85.9 ± 17.05	83.7 ± 9.57	80.5 ± 8.70	90.5 ± 12.6	79.2 ± 5.93
Fasting glucose: 8 weeks (in mg/dl))	97.2 ± 5.44	160.9 ± 19.73 a***	150.8 ± 24.22 a***	142.7 ± 11.76 a***	124.6 ± 8.59 b***, c*	113.1 ± 13.92 a*, b***, c*	81.0 ± 16.07 b***, c***
Fasting glucose: 16 weeks (in mg/dl)	101.1 ± 13.80	190.3 ± 36.85 a***	146.7 ± 21.24 a***	149.9 ± 13.82 a***	146.165± 27.75 b***, c***	139.54 ± 22.29 b***, c***	92.4 ± 11.05
Fasting insulin: 0 days (in ng/ml)	1.71 ± 0.21	1.52 ± 0.22	1.65 ± 0.15	1.61.0 ± 0.27	1.54 ± 0.17	1.58 ± 0.15	1.47 ± 0.06
Fasting insulin: 8 weeks (in ng/ml)	1.56 ± 0.13	3.38 ± 0.27 a***	3.26 ± 0.18 a***	3.27 ± 0.21 a***	2.33 ± 0.10 a***, b***, c***	2.29 ± 0.11 a***, b***, c***	1.47 ± 0.15 b***, c***
Fasting insulin: 16 weeks (in ng/ml)	1.58 ± 0.11	4.93 ± 0.24 a***	3.09 ± 0.24 a***	3.02 ± 0.28 a***	2.86 ± 0.11 b***, c***	2.79 ± 0.31 b***, c*	1.46 ± 0.12

Data are presented as mean ± SD. Differing superscript letters indicate differences between comparison groups where one-way ANOVA; a—compared to NC, b—compared to MS controls, c—compared to groups 3 and 4, * indicates $p \leq 0.05$, and *** indicate $p \leq 0.001$.

Table 3. HOMA-IR at 8 and 16 weeks.

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
HOMA-IR: 8 weeks	1.49 (0.16)	5.36 (0.39) a**	4.85 (0.43) a**	4.58 (0.89) a**	2.83 (0.28) a**, b**,c**	2.55 (0.27) a**, b**,c**	1.19 (0.41) b**,c**
HOMA-IR	1.52 (0.47) b**,c**	8.76 (0.47)	4.42 (0.92) a**, b**	4.30 (0.99) a**, b**	3.93 (1.11) a**, b**	3.66 (.52) a**, b**	1.35 (.234) b**,c**

Data represented as median (IQR)—pairwise analysis results are indicated in terms of significance values with letter, a—compared to group 1, b—compared to group 2, c—compared to groups 3 and 4, *** p value ≤ 0.001, ** p value < 0.01, and * p value < 0.05.

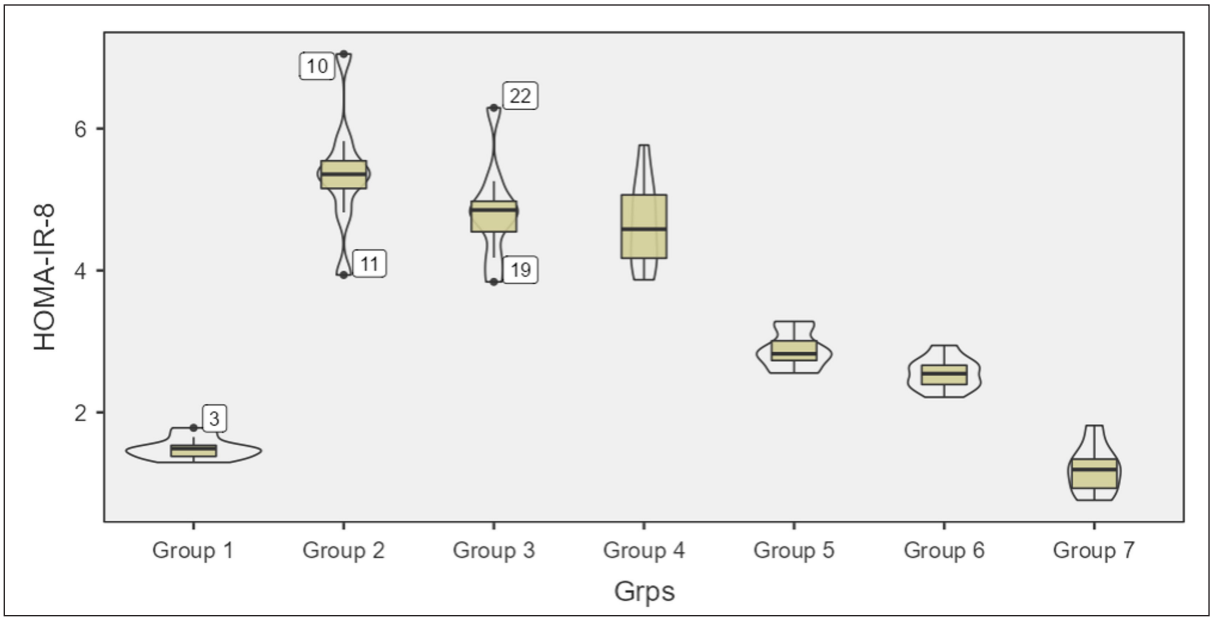

Figure 7. HOMA-IR at 8 weeks in different groups.

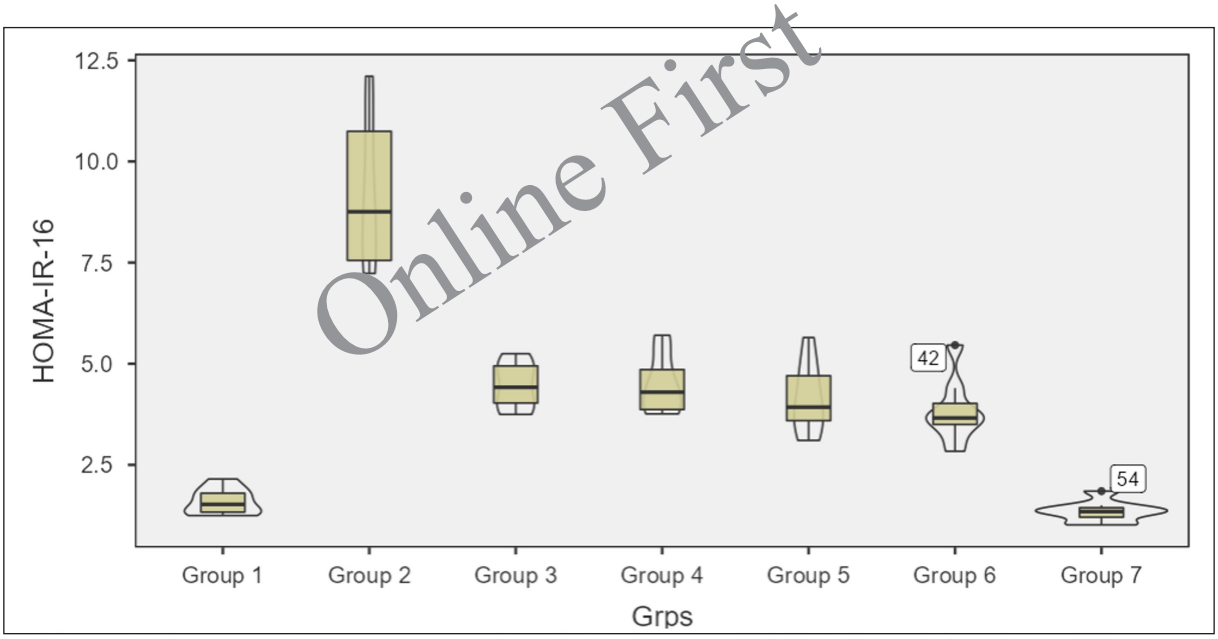

Figure 8. HOMA-IR at 16 weeks in different groups.

total cholesterol in all groups receiving flaxseed extract when compared to metabolic syndrome controls ($p < 0.01$) (Fig. 9).

Serum fasting TAGs were significantly higher in groups receiving HCHF and fructose when compared to NCs (one-way ANOVA Fisher's F [6,49] = 28, $p < 0.001$) and Tukey's post hoc test indicated that high-dose flaxseed extract co-exposure groups demonstrated a significantly lower TAG values when compared to non-flaxseed extract exposure groups ($p < 0.01$). At 16 weeks, total TAG levels differed distinctly among groups (one-way ANOVA Welch's F [6,20.7] = 20.8, $p < 0.001$) with Games–Howell post hoc analysis indicating reduction in total TAGs in all groups receiving flaxseed extract when compared to metabolic syndrome controls ($p < 0.01$) (Fig. 10).

In the first phase of the study, serum fasting HDL-cholesterol levels significantly reduced in all groups receiving HCHF and fructose (one-way ANOVA Fisher's F [6,49] = 24.1, $p < 0.001$) when compared to NCs ($p < 0.001$) but high-dose of flaxseed extract co-exposure prevented the drastic decline in HDL-cholesterol levels when compared to groups not receiving flaxseed extract ($p < 0.01$). At 16 weeks, total HDL-cholesterol levels differed distinctly among groups (one-way ANOVA Welch's F [6,21.5] = 55.7, $p < 0.001$) with

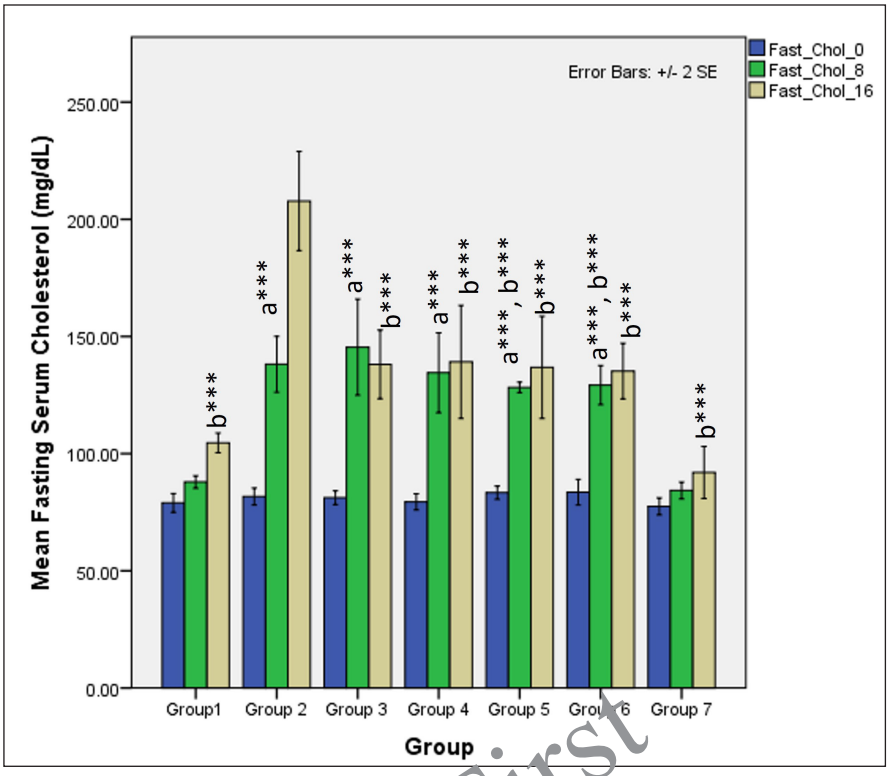

Figure 9. Comparison of fasting total cholesterol at 0 days and 8 and 16 weeks a represents comparison to group 1, b represents comparison to 2, c represents comparison to groups 3 and 4 (one-way ANOVA with post hoc analysis) (*** *p*-value < 0.001, ** *p* < 0.01, * *p* < 0.05).

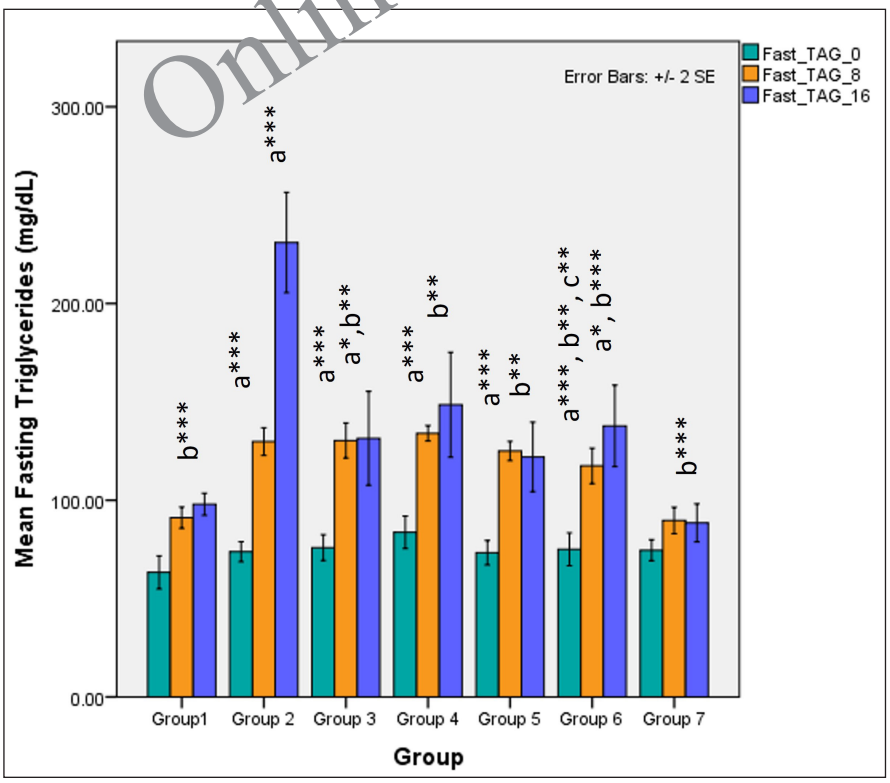

Figure 10. Comparison of fasting triglyceride (TAG's) at 0 days and 8 and 16 weeks a represents comparison to group 1, b represents comparison to 2, c represents comparison to groups 3 and 4 (one-way ANOVA with post hoc analysis) (*** *p*-value < 0.001, ** *p* < 0.01, * *p* < 0.05).

Games–Howell post hoc analysis indicating a reduction in total HDL-cholesterol in all groups receiving flaxseed extract when compared to metabolic syndrome controls ($p < 0.001$) and high-dose flaxseed extract co-exposure groups showing a higher HDL-cholesterol profile than the low-dose post-exposure group ($p < 0.01$) (Fig. 11).

LDL-cholesterol levels during the first 8 weeks differed significantly among different groups (one-way ANOVA Welch's F [6,20.6] = 88.6, $p < 0.001$) with Games–Howell post hoc tests revealing significant elevations in serum LDL-cholesterol receiving HCHF diet and fructose in comparison and among these groups receiving flaxseed extract has lower TAG when compared to metabolic syndrome controls ($p < 0.05$). At 16 weeks, total LDL-cholesterol levels differed significantly among groups (one-way ANOVA Welch's F [6,20.4] = 16.8, $p < 0.001$) with Games–Howell post hoc analysis indicating a reduction in total LDL-cholesterol in all groups receiving flaxseed extract when compared to metabolic syndrome controls ($p < 0.01$) (Fig. 12).

Other metabolic parameters

Flaxseed extract administration significantly improved the plasma adiponectin levels at 16 weeks (one-way ANOVA Welch's F [6, 21.7] = 40.1, $p < 0.001$) (Table 4).

Serum uric acid levels were different during the first 8 weeks (one-way ANOVA Fisher's F [6,49] = 236.8, $p < 0.001$), with higher values observed in groups receiving HCHF and fructose ($p < 0.001$). A high-dose flaxseed extract (group 6) exhibited a lower uric acid than other groups on HCHF and fructose (groups 2, 3, and 4). At 16 weeks, the serum uric acid levels were different among groups (one-way ANOVA Welch's F [6, 21.3] = 90, $p < 0.001$). High-dose co-exposure groups (groups 5 and 6) exhibited a lower uric acid when compared to other groups receiving flaxseed extract for eight weeks and metabolic syndrome controls ($p < 0.05$) (Fig. 13).

Serum TOS was significantly different among groups at 8 weeks (one-way ANOVA Fisher's F [6,49] = 6.28, $p < 0.001$) with significantly higher TOS levels observed in groups only receiving HCHF and fructose (groups 2, 3, and 4) when compared to normal ($p < 0.05$) and flaxseed extract co-administration groups (groups 5 and 6) had significant lower TOS than HCHF and fructose receiving groups (groups 3 and 4) ($p < 0.05$). At 16 weeks, TOS levels were significantly different among groups (one-way ANOVA Fisher's F [6, 49] = 63.93, $p < 0.001$). Among all groups, those receiving HCHF and fructose had higher TOS than NCs ($p < 0.001$). However, high-dose flaxseed extract post-exposure (group 4) and both flaxseed extract co-exposure groups (groups 5 and 6) have lower TOS

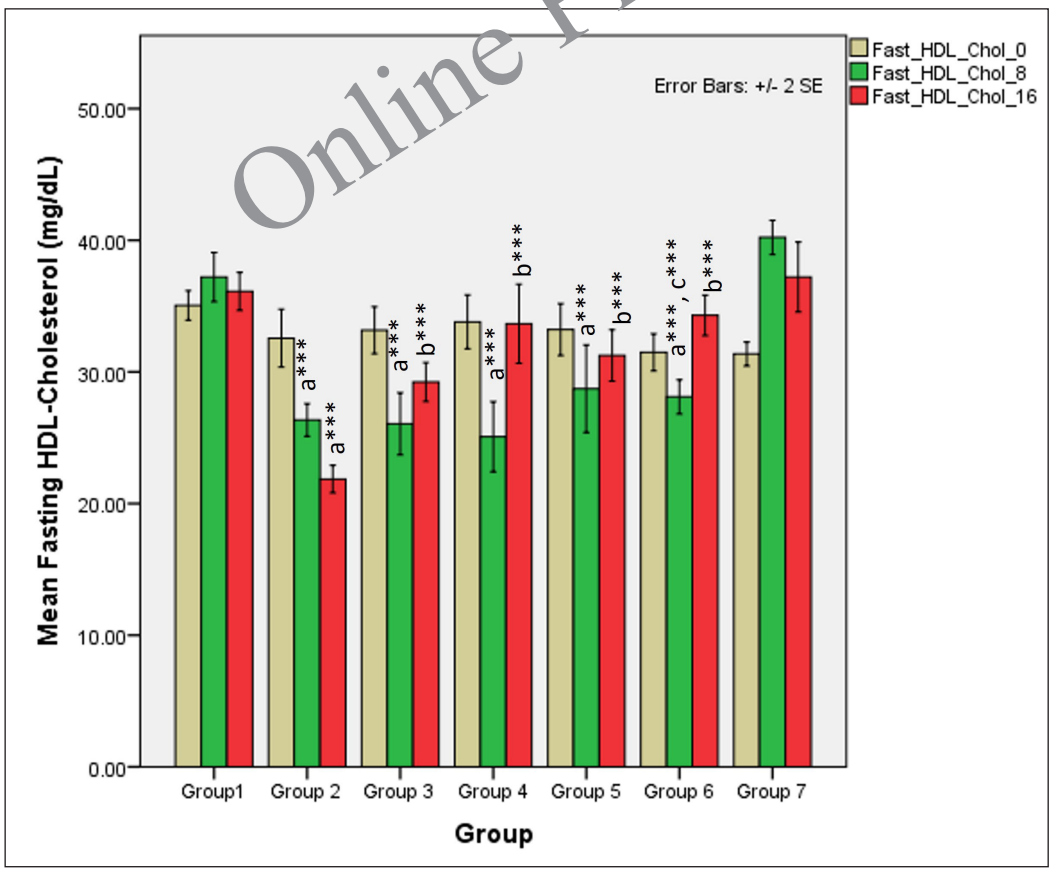

Figure 11. Comparison of fasting HDL-cholesterol at 0 days and 8 and 16 weeks. a represents comparison to group 1, b represents comparison to 2, c represents comparison to groups 2, 3 and 4 (one-way ANOVA with post hoc analysis) (*** p value < 0.001, ** $p < 0.01$, * $p < 0.05$).

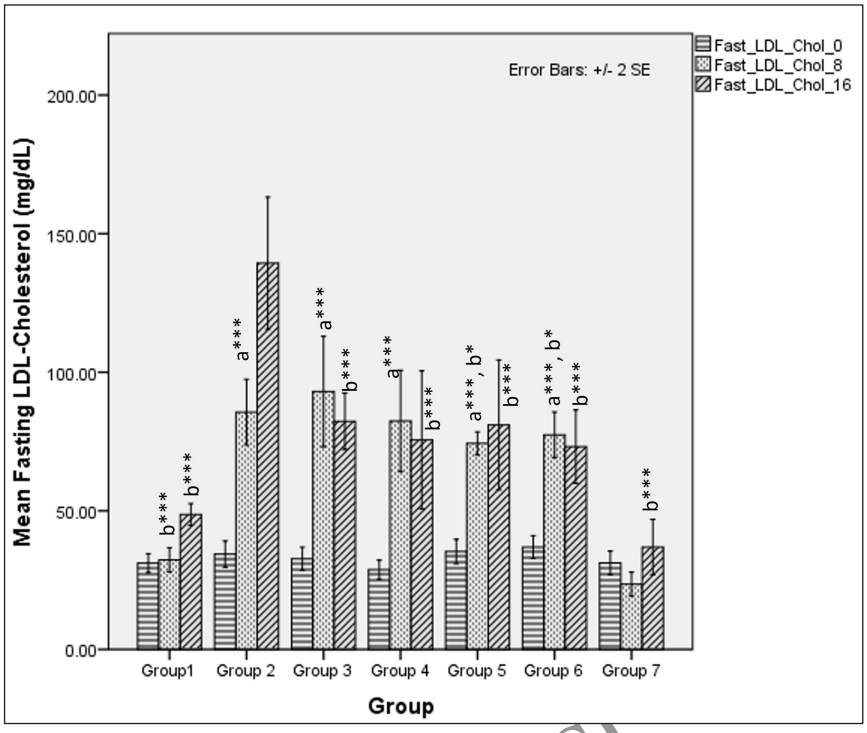

Figure 12. Comparison of fasting LDL-cholesterol at 0 days and 8 and 16 weeks. a represents comparison to group 1, b represents comparison to 2, c represents comparison to groups 2, 3 and 4 (one-way ANOVA with post hoc analysis) (*** p-value < 0.001, ** $p < 0.01$, * $p < 0.05$).

Table 4. Plasma adiponectin levels at different time points

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Plasma adiponectin: 0 days (in µg/ml)	7.19 ± 0.709	6.48 ± 0.669	6.18 ± 0.706	6.12 ± 0.612 a*	6.19 ± 0.505	6.25 ± 0.650	5.89 ± 0.912 a**
Plasma adiponectin: 8 weeks (in µg/ml))	6.26 ± 0.758	4.28 ± 0.644 a***	4.33 ± 0.527 a***	4.15 ± 0.600 a***	4.59 ± 0.443 a***	4.31 ± 0.458 a***	5.85 ± 0.407 a***
Plasma adiponectin: 16 weeks (in µg/ml)	6.12 ± 0.511	2.96 ± 0.385 a***	4.40 ± 0.562 a***, b***,	3.89 ± 0.501 a***, b*,	4.67 ± 0.405 a***, b***,	4.84 ± 0.402 a**, b***,	6.69 ± 0.871, b***,

Data are presented as mean ± SD. Differing superscript letters indicate differences between comparison groups where one-way ANOVA with Tukey's post hoc test; a— compared to NC, b—compared to MS controls, * indicates $p \leq 0.05$, ** indicate $p \leq 0.01$, and *** indicate $p \leq 0.001$.

values than metabolic syndrome (group 2) and the low-dose post-exposure group (group 3) ($p < 0.01$) (Fig. 14).

Serum NO metabolites (nitrate and nitrite) significantly differed among different groups at 8 weeks (one-way ANOVA Fisher's F [6,49] =13.80, $p < 0.001$). Group comparison via Tukey's post hoc test shows that groups receiving HCHF and fructose showed a significant reduction in serum NO metabolite levels as compared to NCs ($p < 0.05$), flaxseed extract administration significantly improved NO metabolites concentrations during the first 8 weeks as compared to groups where flaxseed was not administered ($p \leq 0.05$). At 16 weeks, there was a difference in NO metabolite levels (one-way ANOVA Welch's F [6, 21.5] = 75.4, $p < 0.001$ with Games–Howell post hoc tests) between the groups, indicating that receiving flaxseed extract at a high-dose for 16 weeks has more excellent positive effects on serum NO levels than 8 weeks (Fig. 15).

Histopathological assessment

Histopathological analysis of liver sections indicates the development of steatohepatitis in all the groups administered with HCHF and fructose due to steatotic vacuoles (caused by fat deposition), ballooned hepatocytes, and a few inflammatory foci present (Fig. 16).

Hepatic histopathological scoring of NASH indicated a statistically significant difference between groups (Kruskal–Wallis χ^2 (6) = 36.5, $p < 0.001$, $\epsilon^2 = 0.664$). Pairwise analysis revealed that the difference observed was due to the difference in scores (data represented in Median IQR) between the control groups with normal and flaxseed extract control scores of 0 showing absence of steatotic features as compared to metabolic syndrome controls [group 2: 1.353 (0.714)]. All the groups receiving flaxseeds had an improved median NASH score

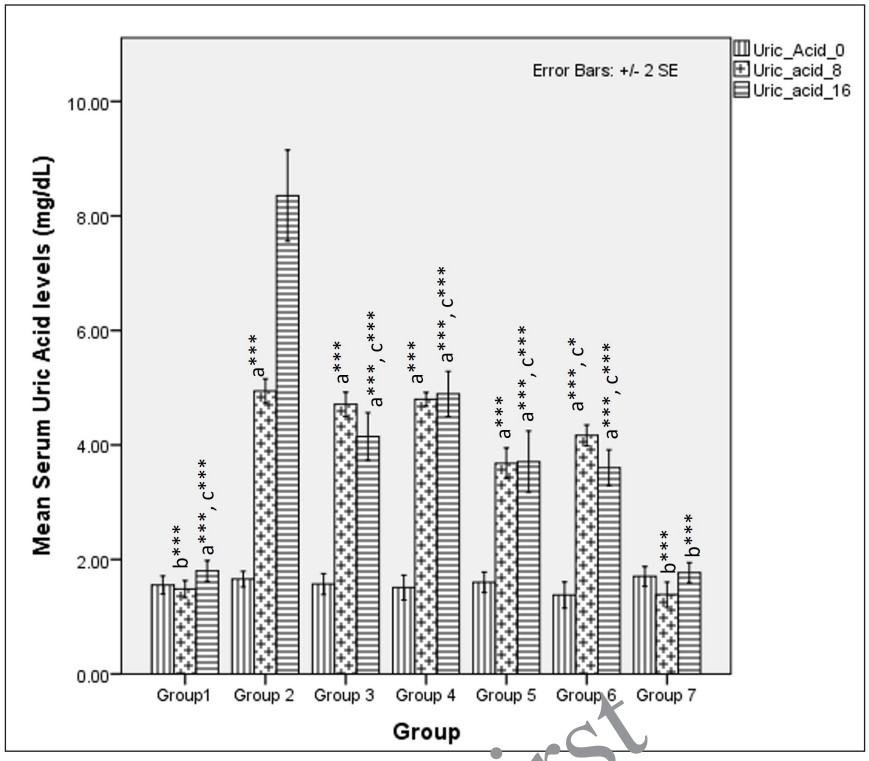

Figure 13. Comparison of serum uric acid levels at 0 days and 8 and 16 weeks. a represents comparison to group 1, b represents comparison to 2, c represents comparison to Group 2, 3 and 4 (one-way ANOVA with post-hoc analysis) (*** p-value < 0.001, ** $p < 0.01$, * $p < 0.05$).

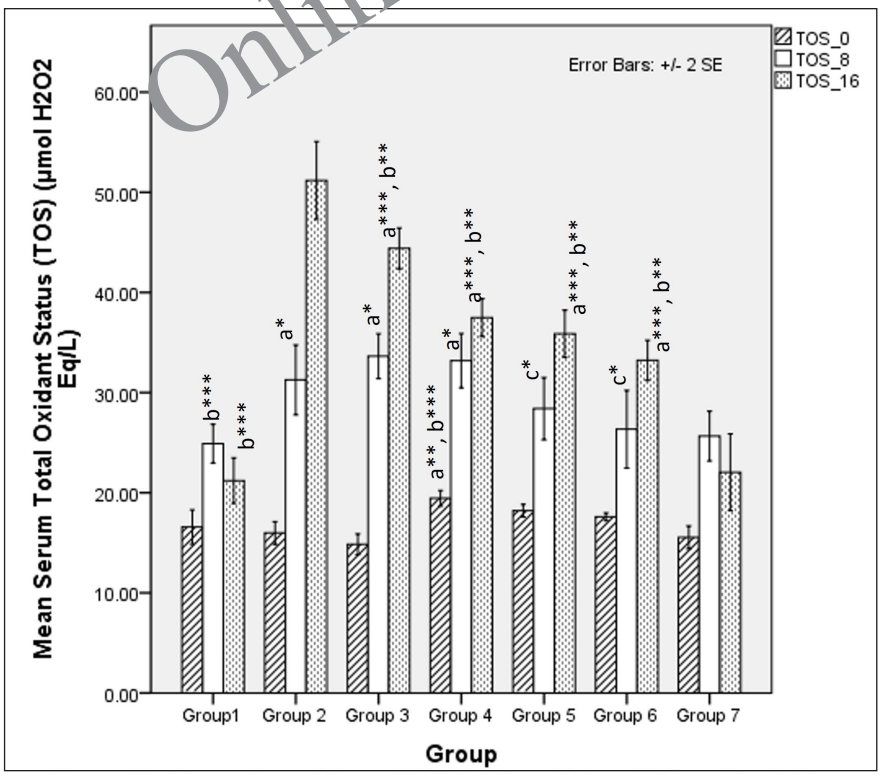

Figure 14. Comparison of serum total oxidant status at 0 days. 8 and 16 weeks. a represents comparison to Group 1, b represents comparison to Group 2, c represents comparison to groups 3 and 4 (one-way ANOVA with post-hoc analysis) (*** p-value < 0.001, ** $p < 0.01$, * $p < 0.05$).

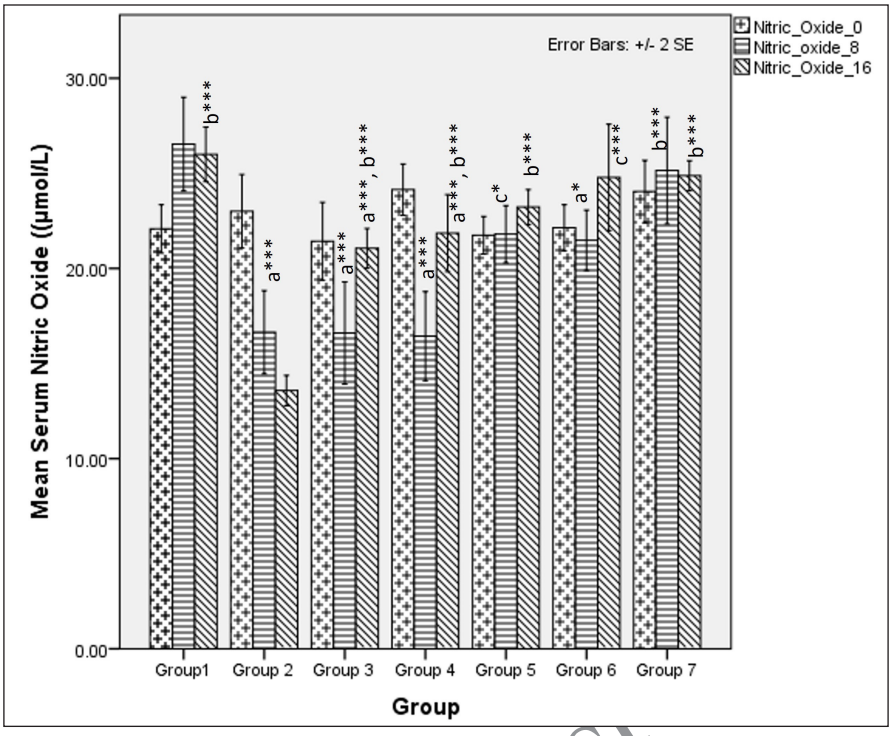

Figure 15. Comparison of serum NO metabolite levels at 0 days and 8 and 16 weeks. a represents comparison to group 1, b represents comparison to 2, c represents comparison to groups 2, 3 and 4 (one-way ANOVA with post-hoc analysis) (*** p-value < 0.001, ** p < 0.01, * p < 0.05).

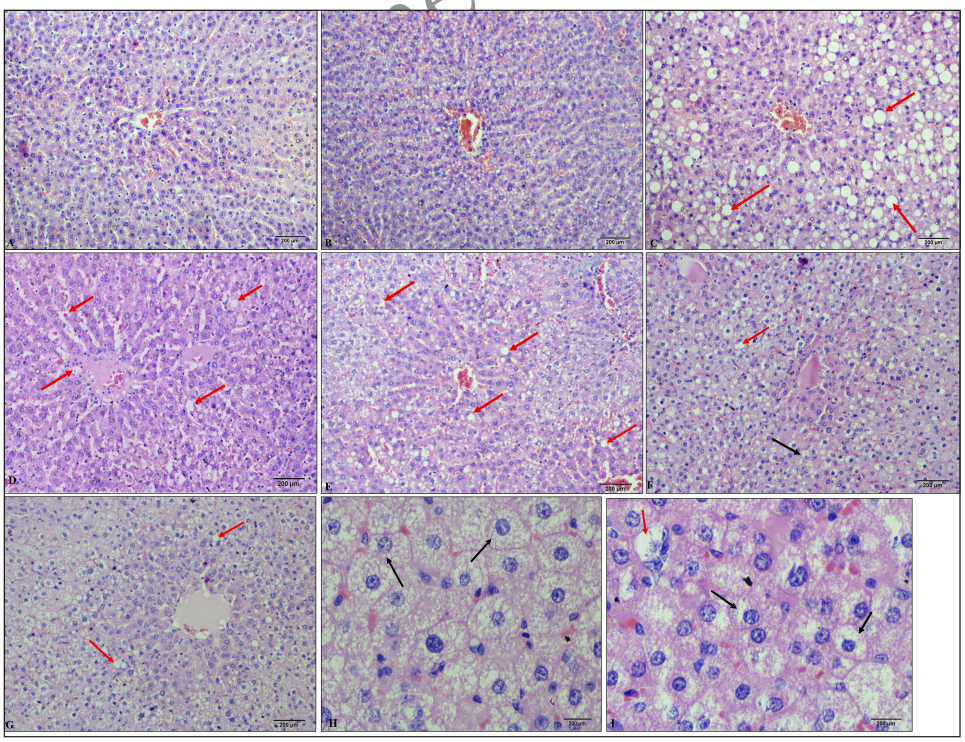

Figure 16. Histological pictures of liver tissue (H & E staining). A: Group 1 (NCs at 100×), B: Group 7 (flaxseed extract controls at 100×), C: Group 2 (metabolic syndrome controls at 100×), D: Group 3 (low-dose flaxseed extract post-exposure at 100×), E: Group 4 (high-dose flaxseed extract post-exposure at 100×); F: Group 5 (low-dose flaxseed extract co-exposure at 100×), G: Group 6 (high-dose flaxseed extract co-exposure at 100×) (red arrows indicate steatotic vacuoles), H & I: Ballooned hepatocytes (at 400×) (Black arrows indicate ballooned hepatocytes where nucleus is pushed to the periphery).

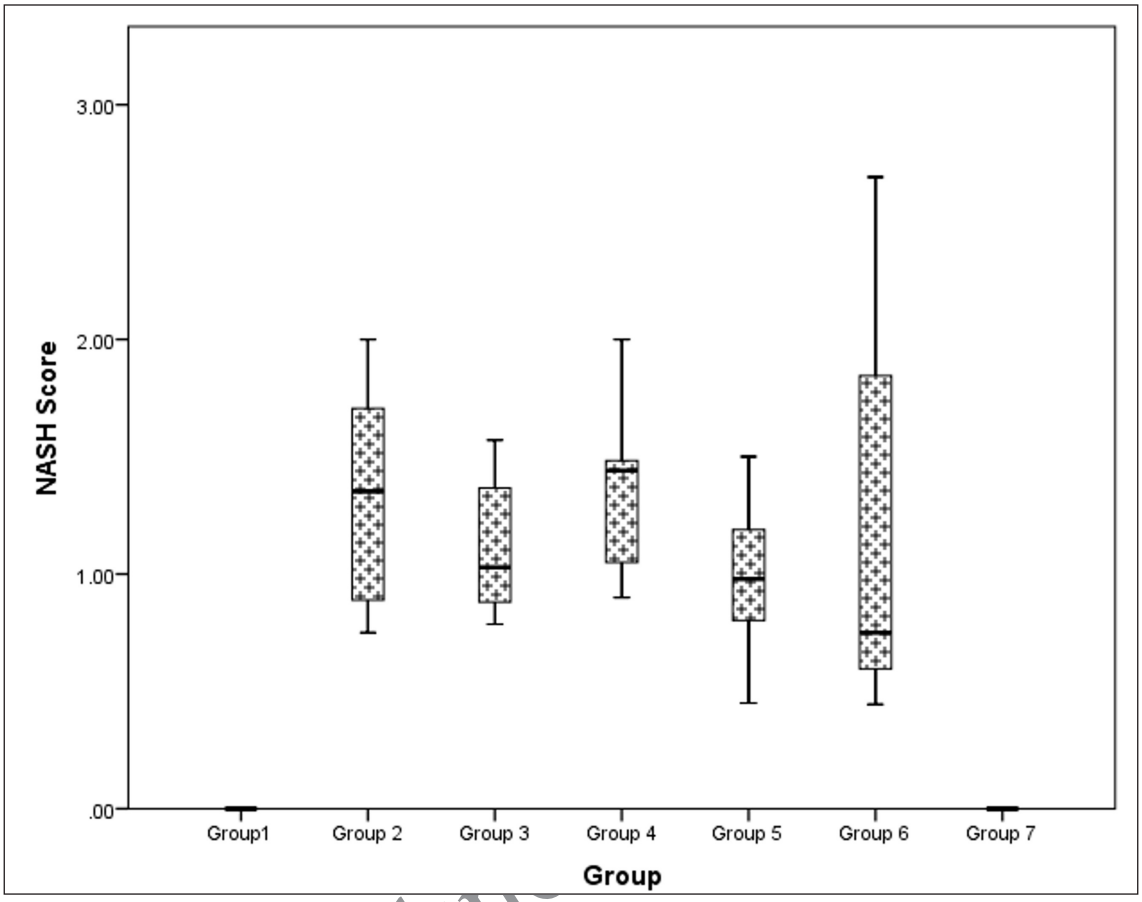

Figure 17. Comparison of NASH (steatohepatosis damage) score at 16 weeks in different groups.

when compared to metabolic syndrome controls, where the groups receiving flaxseed extract for 16 weeks [group 5: 0.980 (0.325)], [group 6: 0.750 (1.056)] showed a better steatohepatitis score than groups receiving flaxseed extract for 8 weeks only [group 3: 1.029 (0.390)], [group 4:1.442 (0.400)]. However, flaxseed extract administration did not inhibit the occurrence of steatohepatitis (Fig. 17).

DISCUSSION

The usage of male rats for the study is to negate the effects of female hormones such as estrogen and their derivatives on the plasma lipids and, in turn, glucoregulatory mechanisms [78–81]. The reason for using whole flaxseeds rather than specific fractions like SDG (Secoisolariciresinol diglucoside) or alpha-linoleic acid fraction is that whole flaxseeds contain a diverse group of biomolecules that can provide a wide range of interactions. These interactions include altering the gut microbiome [35,38,82,83] due to microbial metabolism of indigestible flax lignans, increasing the availability of short-chain fatty acids like butyrate [82], and boosting the expression of adiponectin [34]. By improving gut health and metabolic profile, whole flaxseeds can reduce metainflammation. In groups fed with a high-calorie, high-fat diet and fructose only (group 2: 169 ± 12.,44; group 3: 146.4 ± 17.24, and group 4: 144.1 ± 27.72), change in body weight during the first eight weeks was more as compared to NCs (64.6 ± 4.44), whereas in groups that received flaxseed extracts along with a high-calorie high-fat diet and fructose, the change in body weight was much lesser (group 5: 80 ± 17.24; group 6: 70.5 ± 14.06). During the next 8 weeks in the two post-exposure groups, the gain in body weight was significantly reduced compared to metabolic syndrome controls due to flaxseed extract supplementation. The results indicate that flaxseed extract significantly inhibits body weight gain even when fed on a hypercaloric high-fat diet, consistent with earlier studies [84,85]. The effects of flaxseed extract on abdominal obesity were also one of the crucial observations where flaxseed extract administration significantly prevented the increase of AC when compared to animals without flaxseed extract when both were administered a high-calorie, high-fat diet, which is in opposition to previous studies [34]. The administration of flaxseed extract affects plasma adiponectin concentration, and this may be one of the mechanisms by which flaxseed extract regulates body weight and visceral obesity, as evident from reduced visceral adiposity index value among groups with flaxseed extract administration for 8 and 16 weeks [86,87]. Adiponectin, an adipokine released from adipocytes, significantly affects systemic homeostasis via its effects on different metabolic pathways in different organs, such as the activation of AMP-activated protein kinase (AMPK) in skeletal muscles and adipocytes, which may increase fatty

acid oxidation pathways and induce beiging of white adipose tissue and brown adipocyte thermogenesis, all of which may promote energy expenditure and reduce adiposity [88–91]. Studies have also shown that circulating levels of adiponectin inversely correlate with body-fat mass [89], and flaxseed extract-induced elevations in adiponectin may account for the reduced adiposity and visceral fat index observed in the groups receiving flaxseed extract. In our study, introduction of a high-calorie, high-fat diet led to accumulation of lipids in tissues (increasing body fat index), which affects the serum adiponectin levels. However, in the groups that received flaxseed extract in conjunction with a high-calorie, high-fat diet, had a higher adiponectin level which is in line with the findings of previous studies [33,92,93]. Among the groups, receiving a high-calorie, high-fat diet and fructose in conjunction with flaxseed extract, the co-exposure group presented elevated levels of circulating adiponectin at 16 weeks, and the high-dose group presented the highest concentration, indicating that high-dose co-exposure may significantly increase adiponectin levels. Adiponectin also plays a major role in glucose metabolism and insulin sensitivity where the binding of adiponectin to its cognate receptors can induce the translocation of insulin-sensitive GLUT-4 receptors to the membrane, and increase the insulin signaling pathways via the activation of insulin receptor substrates [87,88]. Adiponectin can influence the transcription of insulin-synthesizing genes and exocytosis of insulin-containing granules from pancreatic beta-cells, all of which may play a role in improving insulin resistance [88,94,95]. In our study, the administration of a high-calorie, high-fat diet with fructose significantly increased plasma fasting glucose and insulin levels and increased insulin resistance. However, the administration of flaxseed extract reduced the plasma glucose levels with the most significant reductions in fasting glucose, insulin, and HOMA-IR observed in co-exposure groups when compared with those in the non-flaxseed and post-exposure groups. Among the co-exposure groups, the high-dose co-exposure group presented the greatest reduction in insulin resistance index (HOMA-IR), indicating its better efficacy. The effect of flaxseed extract on fasting plasma glucose levels also presents a promising area of interest since, in our study, we have seen that flaxseed extract lowers the fasting plasma glucose levels even in the event of continued high-calorie, high-fat, and fructose consumption. However, it fails to reduce the fasting glucose within normoglycemic limits (<7.2 mmol/L). Still, it lowers glucose levels compared to metabolic syndrome controls, which can be attributed to elevated insulin sensitivity and peripheral utilization [26]. The elevated insulin sensitivity in our study also explains the lower fasting insulin levels in the groups treated with the flaxseed extract than in the non-flaxseed extract groups, with maximum reductions in fasting insulin levels observed in the high-dose co-exposure group [96].

With regard to plasma lipids, during the first 8 weeks, the total cholesterol was lower in groups receiving a high-dose of flaxseed extract (1.0g/kg/bd.wt/day) when compared to groups receiving high-calorie, high-fat diet and fructose only, highlighting the hypolipidemic effects of flaxseed extract which is in line with some of the studies [97]. During the next 8 weeks, when flaxseed extracts were introduced into already hyperlipidemic hyperglycemic metabolic syndrome parametric groups, it reduced fasting total cholesterol. In those groups already on flaxseed extract, continuation of flaxseed extract maintains the total cholesterol levels with the continuation of a high-calorie, high-fat diet, which is in line with one of the other studies [84], which may be attributed to reduced hepatic cholesterol synthesis and inhibition of entero-hepatic bile acid absorption [98]. The levels of HDL-cholesterol in groups receiving high-calorie, high-fat, and fructose at the end of the first 8 weeks were reduced compared to NCs. However, groups co-receiving flaxseed extract showed a lesser reduction in HDL-cholesterol levels, with a higher dose of flaxseed extract providing a better HDL-cholesterol profile than those with a low dose. During the next phase of 8 weeks, when Wistar rats with lowered HDL-cholesterol were initiated on a diet of flaxseed extract, the levels of HDL-cholesterol were higher than levels at 8 weeks, indicating that flaxseed extract promotes the production of HDL-cholesterol via Paraoxonase-1 (PON-1) action in promoting a better reverse cholesterol transport [99]. The levels of LDL-cholesterol during the first 8 weeks of high-calorie, high-fat, and fructose feeding increased probably due to increased hepatic lipogenesis stimulation by fructose. However, in groups receiving flaxseed extract concurrently, the levels of LDL-cholesterol were lower. In the succeeding 8 weeks, when these hypercaloric high-fat fed rats were initiated on flaxseed extract, they showed a reduction in LDL-cholesterol level, which could be attributed to increased hepatic expression of LDL-receptor mediated by flaxseeds as indicated in rodents [98]. Recent evidence has indicated that adiponectin levels also play a key role in dyslipidemia, by reducing the levels of serum TAG, total cholesterol, and LDL-cholesterol [100–102]. Adiponectin and HDL-cholesterol share an intricate relationship in which adiponectin prevents a reduction in HDL-cholesterol levels, which in turn increases circulating adiponectin levels [101]. Therefore, the increase in HDL-cholesterol level and subsequent reduction in TAG, total cholesterol, and LDL-cholesterol levels can be attributed to increased serum adiponectin levels stimulated by flaxseed extract. Compared with the non-flaxseed groups, the groups that received the flaxseed extract presented an improved lipid profile, and compared with the low-dose groups, the high-dose flaxseed presented a greater increase in HDL-cholesterol.

The serum levels of uric acid were elevated upon ingestion of fructose diets due to the metabolic breakdown of fructose-generating uric acid [103,104], which positively correlates with metabolic syndrome in rodents and humans [105–107]. Our study found that uric acid levels at 8 weeks are elevated in all the groups, probably due to the combined effects of fructose feeding and hyperglycemia-induced kidney dysfunction. High-dose flaxseed extract supplementation lowered uric acid levels at the end of 16 weeks, similar to STZ models of T2DM [26]. This reduction in uric acid levels may be attributed to the improvement of hyperglycemia-induced renal function [108] and AMPK activation by flaxseed polysaccharides [35], which, in turn, may provide a protective function against AMPK-hyperuricemia interaction-mediated renal dysfunction [109]. The levels of TOS in our study also indicate the anti-oxidative properties of flaxseed extract wherein

high-calorie, high-fat, fructose-induced oxidative stress is reduced only in the high-dose flaxseed extract administration group as compared to other groups, and at the end of 16 weeks, as compared to metabolic syndrome controls all other groups show a lower TOS level. However, the values at 16 weeks are higher than those at 8 weeks, indicating that flaxseed extract administration positively reduces oxidative stress by inducing the antioxidant genes [108] and due to the presence of ALA in flaxseeds. The importance of oxidative stress and antioxidants in the maintenance of metabolic homeostasis and prevention of diet-induced obesity has been elucidated in a recent study that revealed the reversal of high-fat diet-induced alterations in tyrosine phosphorylation by the antioxidant butylated hydroxyanisole [110].

The levels of NO metabolites were reduced upon administration of a high-calorie, high-fat diet-mediated obesity and hyperglycemia-induced oxidative stress [111,112], which initiates endothelial dysfunction resulting in hypertension. The reduction in NO levels may be due to a reduction in adiponectin levels, since it can promote NO via inducible NO synthase enzyme [88]. The levels of NO metabolites may also be affected by insulin resistance since there is an inverse relationship between NO metabolites and HOMA-IR [113–115]. Since flaxseed extract improves serum adiponectin levels and insulin sensitivity, it may be collectively responsible for increasing serum NO metabolites which may be protective since it can effectively reduce endothelial dysfunction and promote vasodilation both of which reduce the risk of hypertension, as observed in individuals with MetS [116]. Our results demonstrated that, among the groups that received the flaxseed extract, the high-dose co-exposure group showed the highest levels of serum NO metabolites as compared with the other groups that received flaxseed extract, suggesting a better endothelium-protective effect.

The liver histopathological analysis also reveals that flaxseed extract has a minor hepatoprotective effect and reduces the magnitude of steatohepatitis, which can be attributed to flaxseed-induced liver lipid metabolism changes leading to reduced hepatic lipid accumulation [117] and antioxidant properties of flaxseed extract (which include increasing liver reduced glutathione levels) [118]. However, they cannot prevent the occurrence of non-alcoholic fatty liver due to fructose insult, since the steatohepatitis scores (NASH scores) in the groups receiving flaxseed extract were not low, as observed in the normal or flaxseed extract control groups. However, the median NASH scores in the groups receiving flaxseed extract were lower than those in the non-flaxseed groups. Compared with those in the post-exposure groups, the median NASH scores in the flaxseed co-exposure groups improved, with the lowest scores observed in the high-dose co-exposure group. These observations indicate that flaxseed extract improves steatohepatosis scores and ameliorates or reduces the intensity of fructose-induced hepatic fat deposition but does not confer complete protection against fructose-induced steatohepatosis. The probable reason for this observation may be the flaxseed extract-induced increase in serum adiponectin which is known to downregulate hepatic gluconeogenic and *de novo* lipogenic genes and upregulate fatty acid oxidation [88], all of which might collectively regulate hepatic lipid accumulation. However, the continuation of the addition of fructose along with flaxseed extract which is known to promote hepatic *de novo* lipogenesis [119] may be responsible for the absence of complete protection.

The probable reason for the delay in changes in some of the parameters seen only after eight weeks of flaxseed extract administration or only in cases of high-dose flaxseed extract may be due to the reason that adequate serum levels of beneficial metabolites like ALA from flaxseeds may occur only in high-dose or more-extended duration scenarios [120,121]. The high-dose co-exposure works best as seen in our study, still, a detailed pharmacokinetic or pharmacodynamic study would be essential. We have seen in our study that high-dose co-exposure provides the least reductions in adiponectin levels which could be one of the reasons, since high-fat high-calorie diets impair adiponectin gene transcription but components of flaxseeds induce adiponectin expression, and high concentration and exposure along with HCHF may prevent the HCHF and fructose-induced adiponectin repression.

Other natural approaches in metabolic syndrome treatment from different herbal or dietary components include nutraceuticals such as garlic (lowering blood pressure with no effects on adiposity) [122], blueberry, and polyphenols (improve glucose sensitivity but shows no mentioned effect on adiposity, serum lipids) [122], ginseng (improved fasting glucose and lipids, with no effect on blood pressure) [122], pomegranate (reducing blood pressure but no effects on adiposity, glucose and lipid levels) [122]. Some studies have included compounds such as quercetin (glucose sensitivity enhancers with no effect on blood pressure) [122], vitamins B3 and D (which only target metabolic syndrome indicators such as fasting lipids for B3 and HOMA-IR, SBP, and DBP for vitamin D) [122]. Compared to other nutraceuticals, green tea has shown the best results in targeting the majority of metabolic syndrome indicators, namely, reducing adiposity, blood pressure, fasting glucose, and lipids [122]. However, the effects of green tea on adiponectin and leptin are still not clearly elucidated.

Flaxseed supplementation improves glycemic control and insulin sensitivity among prediabetes and T2DM human subjects, but many studies have not been done in metabolic syndrome patients targeting glycemic control, dyslipidemia, or blood pressure [123,124]. Some studies conducted on metabolic syndrome subjects have different varied doses, different routes of administration, and usage of flaxseed oils [124] in some studies, yielding conflicting results with some studies indicating beneficial effects on glycemic control or lipid levels. Human clinical trials need to be more decisive about the maintenance of doses and administration routes and look at other parameters such as NO, PON-1, Apo-A1, and lipoprotein (a) levels in metabolic syndrome and how they are affected due to flaxseed administration. Our study has indicated a protective effect of flaxseeds in co-administration of flaxseeds and high-calorie, high-fat, and fructose diets. However, more studies need to be conducted to elucidate these protective effects in the co-consumption of flaxseeds and Western-based diets to decipher the mechanistic basis of the attenuative potential of flaxseeds.

CONCLUSION

Whole flaxseed extract co-administration at high doses improves blood glucose, lipid profiles, and anthropometric indices in high-calorie, high-fat fructose-fed rats mimicking metabolic syndrome symptoms and helps in mitigating the harmful effects of hypercaloric high-fat fructose diets.

LIMITATIONS OF THE STUDY

The limitations of the current study can be identified as the absence of a genomic approach like estimation of liver AMPK gene expression or the genomic expression of lipogenic genes or microRNAs in liver and adipose tissue to elucidate the probabilistic mechanistic action of flaxseeds in mitigating the effects of diet-induced obesity or fructose feeding. The future prospects of the study might include an epigenomic approach investigating the diet-metabolism interactions that may occur due to flaxseed consumption.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Manipal Academy of Higher Education, Manipal, India (Approval No.: IAEC/KMC/103/2019).

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER'S NOTE

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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