**Original Paper****Linseed oil supplementation improves altered lipid metabolism and insulin resistance in induced obese rats**

Esraa M Seliema*, Mohamed E Azab, Randa S Ismaila, Abeer A Nafeaa

Department of Physiology, Faculty of Veterinary Medicine, Benha University, Egypt

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ABSTRACT

The present work was performed to elucidate the effect of linseed oil supplementation on metabolic disorders in induced obese male albino rats. Thirty male Albino Wister rats were used in this investigation. They were categorized into two groups; 10 rats received a control-diet, and 20 rats were supplied a high-fat diet for two months till the induction of obesity. Then, rats were split into 3 groups (10 rats each). Group I was maintained on a control diet. Group II was maintained on HFD only. Group III received HFD with 30% linseed oil for further two months. At the end of the experiment, the rat's body and organs weights were assessed. Blood samples and adipose tissue were obtained for analysis. The results revealed that linseed oil supplementation caused a substantial ($P < 0.05$) reduction in serum TC, TAG, LDL-C, VLDL-C, glucose, insulin levels, and HOMA-IR. However, HDL-C and adiponectin levels showed a significant ($P < 0.05$) increase when compared with the HFD group. Linseed oil supplementation also caused significant down regulation of retinol-binding protein 4 (RBP4) gene expression, however, the GLUT4 gene showed significant upregulation. From the obtained results it could be concluded that linseed oil has an ameliorative effect on altered lipid profile caused by obesity. In addition, linseed oil caused improvement of insulin resistance in addition to the pro and anti-inflammatory biomarkers

1. INTRODUCTION

Obesity and overweight are on the rise all over the world (Karri *et al.*, 2019). Obesity is caused by an energy imbalance resulting from an excess of caloric intake compared to energy expenditure (Wang *et al.*, 2014). It is a condition characterized by low-grade chronic inflammation induced disruption in inflammation resolution (Bashir, Ali and Khan, 2015).

Obesity has been related to the elevation of adipose tissue macrophage infiltration, which could contribute to adipocyte hypertrophy. The infiltration of macrophages into adipose tissue, as well as their polarizing toward a pro-inflammatory state, has been convicted at the beginning of obesity-related disorders in both humans and rodent models (Jung and Choi, 2014). The mechanism of macrophage polarization includes the conversion of adipose tissue macrophages (ATMs) from M2 macrophages (anti-inflammatory) that release adiponectin, IL-10, and other homeostatic mediators to M1 macrophages (pro-inflammatory) that secrete a plethora of pro-inflammatory cytokines as RBP4 sustain an inflammatory environment (Jung and Choi, 2014).

Finding cost-effective alternatives to obesity management is becoming increasingly critical. New evidence about the use of omega-3 fatty acids as anti-obesity and anti-inflammatory agents has emerged (Wang *et al.*, 2014). Higher dietary omega-3 fatty acid consumption is implicated as a cause of diminishing the proliferation of

lymphocytes, pro-inflammatory cytokines, and M1 states in ATMs (Baranowski *et al.*, 2012).

Linseed (*Linum usitatissimum L.*) is the richest plant source of omega-3 fatty acids include α -linolenic acid (ALA) that can be elongated and desaturated to generate eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) (Baranowski *et al.*, 2012). The benefits of linseed oil against disorders of lipid metabolism and its impacts on insulin resistance and type 2 diabetes have been investigated by a previous study (Yu *et al.*, 2018).

Therefore, this study was conducted to study the expected or possible protective effect of linseed oil as a rich source of omega-3 fatty acids on metabolic disorders such as hyperlipidemia, insulin resistance, pro- and anti-inflammatory biomarkers. This was achieved by studying the impact of linseed oil on body and organs weights, lipid profile, insulin resistance as well as gene expression of RBP4 and GLUT4.

2. MATERIAL AND METHODS**2.1. Animal of the experiment:**

Thirty male Albino Wister rats, 5-6 weeks old and average body weight 170 ± 10 g were used in this experiment. Rats were purchased from Laboratory Animals Research Center, Faculty of Veterinary Medicine, Benha University, Egypt. Animals were housed in separate stainless-steel cages and maintained under a humidity-controlled environment with a Light-dark cycle of 12 hours, and steady temperature

* Correspondence to: israa.magdy18@fvvm.bu.edu.eg

ranged from +22 to +28 °C. Food and water were freely accessible. The Animals were acclimatized to the laboratory condition for the first week before the experiment. During this period, A balanced control basal diet was given.

The experiment protocol was approved by the Animal Care and Use Ethics Committee of Faculty of Veterinary Medicine, Benha University, Egypt (approval number: BUFVTM 02-09-21).

2.2. Experimental design:

Pursuing the period of acclimation, the rats were assigned into 3 equal groups (10 rats per group). The first group was given a basal diet as control and the other two groups were nourished high-fat diet with 40% beef tallow for 8 weeks to induce obesity. The overall composition of the two diets for rats is shown in Table 1.

Subsequent obesity induction, the rats were allocated randomly into 3 weight-matched groups (n=10/group) placed in individual cages to be classified as follows: Group I (Control group): rats have been given control basal diet. Group II (HFD group): Obese rats received a high-fat diet only. Group III (HFD + LO): Obese rats were fed HFD supplemented with 299.2 g linseed oil/kg of diet (30% of linseed oil supplementation) (Yu *et al.*, 2019). This protocol lasted eight weeks and the proportion of beef tallow replaced by linseed oil was determined based on the investigation done by (Yu *et al.*, 2019).

All rats were examined daily, and body weights were monitored weekly.

Table 1 The composition of experimental diets

Ingredients	Standard Diet (AIN-76A diet #100000)		High Fat Diet (AIN-76A diet #101556)	
	G	Kcal	G	Kcal
Casein	200	720	200	720
Beef tallow	0	0	400	3600
Methionine	3	12	3	12
Starch	150	540	150	540
Sucrose	500	2000	150	600
Cellulose	50	0	50	0
Corn oil	50	450	0	0
Salt mixture	35	30.8	35	30.8
Vitamin mixture	10	39	10	39
Choline bitartrate	2	0	2	0
Total	1000	3791.8	1000	5541.8

2.3. Sampling:

At the end of the experiment, rats were obligated to fast for the full evening. Following fasting, they were anesthetized and executed. The blood samples were collected immediately in serum separator gel blood collection tubes. These tubes were centrifuged for 20 minutes at 3000 rpm to obtain the serum for the biochemical analysis and stored at -20 °C until the time of analysis. Fresh liver, spleen, kidney, and heart were excised and weighed quickly. Moreover, epididymal adipose tissue samples were excised, with one portion of fresh adipose tissue being fixed for histopathological examination and the other being quickly frozen at -80 °C for real-time PCR analysis.

2.4. Serum biochemical biomarkers:

Serum lipids, adipokines, and metabolic biomarkers were evaluated. The level of total cholesterol (TC) according to Allain *et al.*, (1974), triacylglycerol (TAG) according to Fossati and Prencipe, (1982), high-density lipoprotein-cholesterol (HDL-C) according to Lopes-Virella *et al.*, (1977), low-density lipoprotein-cholesterol (LDL-C), very low-density lipoprotein-cholesterol (VLDL-C) according to Friedewald *et al.*, (1972), and glucose according to Trinder, (1969) were examined by enzymatic colorimetric method

using commercially available kits purchased from Biodiagnostic company, Cairo, Egypt in accordance with the manufacturers instructions. Levels of insulin and adiponectin were measured with specific (relevant) ELISA kits (Abnova Corporation, Taipei, Taiwan, and BioVision, Milpitas, California, USA). Insulin resistance was detected by using the HOMA method applying the formulae below:

Insulin resistance (HOMA-IR) = fasting insulin ($\mu\text{U/ml}$) \times fasting glucose (mmol/l)/22.5 (Wallace, Levy and Matthews, 2004).

2.5. Histological study:

Adipose tissue was excised and fixed immediately in 10 % neutral buffered formalin used then submerged in paraffin. Tissue blocks that had been formalin-fixed and paraffin-submerged were sliced to a thickness of 4 mm then Hematoxylin and eosin staining (H&E) occur using standard histopathology procedures. The sections were imaged at 100X and 400 X magnifications.

2.6. Total RNA isolation and real-time quantitative RT-PCR:

Total RNA of adipose tissue was extracted by using Gene JET RNA Purification Kit (Thermo Scientific, Waltham, Massachusetts, U.S.) according to the instructions provided by the manufacturer. From the extracted RNA, cDNA was generated using TOPscript™ cDNA Synthesis Kit (enzymatics, Daejeon, South Korea). Then the RNA expression levels of RBP4 and GLUT4 genes were investigated by Rotor-Gene Q 5plex system (QIAGEN, Hilden, Germany) using a TOPreal™ qPCR 2X PreMIX (SYBR Green with low ROX) (enzymatics, Daejeon, South Korea) following the manufacturer's directions. the sequences of the primers used for PCR were shown in Table 2.

The RT-PCR conditions included an initial denaturation at 95 °C for 15 min, followed by 45 PCR cycles, with each cycle composed of 10 s at 95 °C, 15 s at 65 °C, and 30 s at 72 °C. After that, a melting curve study is conducted. The mRNA expression levels of RBP4 and GLUT4 were normalized to the expression level of GAPDH. The results were elucidated as fold changes when compared to the control group.

Table 2 Quantitative real-time RT-PCR primers

Gene		Primers (5' → 3')
RBP4	Forward	TGATCGTCCACAACGGTTAC
	Reverse	GAGCTGAAGACTGAGAGCTAATC
GLUT4	Forward	CCATAGGAGCTGGTGGTCAATAC
	Reverse	TCGCCAGCTCGCTCTACTAAG
GAPDH	Forward	GGTGTGAACCATGAGAAGTATGA
	Reverse	GAGTCTTCCACGATACCAAAG

2.7. Statistical analysis:

All statistical analyses were performed by using SPSS Statistics version 26 (IBM, SPSS Inc., Chicago, USA). The substantial difference between groups was evaluated by one-way ANOVA using the Duncan test as a post hoc. The results are displayed as means \pm SE, with significance considered at $P < 0.05$.

3. RESULTS

3.1. Effect of Linseed oil on the weight of the body and organs:

The changes in body, heart, liver, kidney, and spleen weights in animals given the control, HFD, or HFD with linseed oil are exhibited in Table 3. The HFD group

revealed a substantial elevation in live body weight, heart, liver, kidney, and spleen weights compared to the control group. Rats fed HFD with linseed oil supplementation revealed a significant decline in body, heart, liver, kidney, and spleen weights when compared with the HFD group. There was a marked increase in body, heart, liver, kidney, and spleen weights of linseed oil supplemented rats compared with control rats.

Table 3 Effect of linseed oil supplementation on Body, Heart, Liver, Kidney, and liver weights of HFD induced obese rats.

Parameters	Experimental groups		
	Control	HFD	HFD+LO
Body Weight (g)	298.50±3.45 ^c	576.88±6.40 ^a	362.00±2.75 ^b
Heart Weight (g)	1.25±0.05 ^c	2.29±0.04 ^a	1.64±0.03 ^b
Liver Weight (g)	7.65±0.43 ^c	16.18±0.53 ^a	11.59±0.19 ^b
Kidney Weight (g)	1.51±0.05 ^c	2.97±0.05 ^a	2.06±0.07 ^b
Spleen Weight (g)	0.76±0.05 ^c	2.06±0.08 ^a	1.05±0.05 ^b

HFD; high-fat diet and HFD + LO; high-fat diet with linseed oil. Means within the same row having different superscripts are significantly different at $p < 0.05$

3.2. Effect of linseed oil on serum metabolic parameters:

The effect of linseed oil on metabolic parameters of serum is described in Fig. 1A, 1B, 1C, 1D, 1E, 2A, 2B, 2C, and 2D, respectively. HFD group manifested a significant elevation in serum TC, TAG, HDL-C, LDL-C, VLDL-C, glucose, insulin, and HOMA-IR, as demonstrated in Fig. 1A, 1B, 1C, 1D, 1E, 2A, 2B, and 2C, respectively, when compared with those in controls. While compared with the HFD group, the serum adiponectin level of the control group substantially increased as revealed in Fig. 2D.

Linseed oil substantially reduced the HFD-induced rise in TC, TG, LDL-C, and VLDL-C levels in comparison with the HFD group. Although linseed oil caused a remarkable reduction in these parameters, HFD + LO group revealed a significant increase in these parameters when compared with the control group. On the contrary, a dramatic upregulation of HDL-C level in the serum of the HFD + LO group was observed in comparison with HFD and control groups.

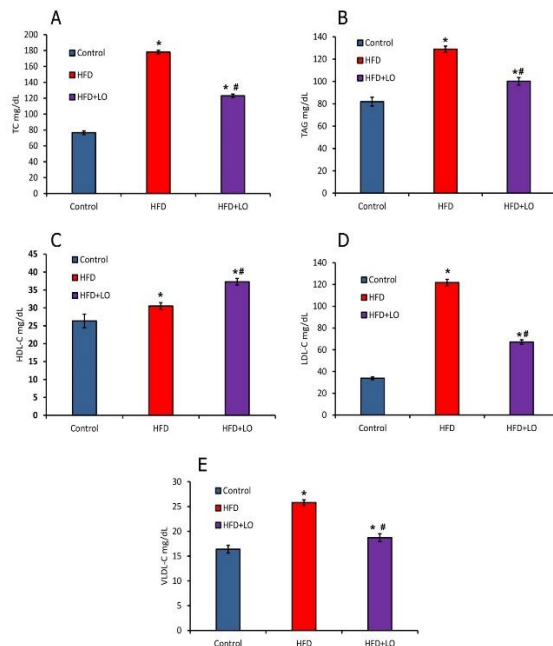


Figure 1. Effect of linseed oil on serum lipid profile (A) total cholesterol (TC), (B) triacylglycerols (TAG), (C) high density lipoprotein cholesterol (HDL-c), (D) low density lipoprotein cholesterol (LDL-c), and (E) very low-density lipoprotein cholesterol (VLDL-c) concentration (mg/dl) in induced obese rats fed HFD for 8 weeks. Data are presented as the mean \pm SE, n=8. * $p < 0.05$, compared with control; and ** $p < 0.05$, compared with HFD.

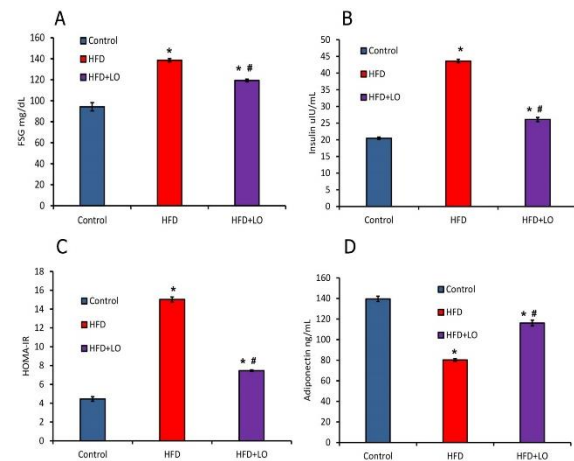


Figure 2. Effect of linseed oil on the serum level of (A) fasting serum glucose (FSG) (mg/dl), (B) insulin (uIU/mL), (C) homeostasis model assessment-insulin resistance (HOMA-IR) (D) adiponectin (ng/mL) in induced obese rats fed HFD for 8 weeks. Data are presented as the mean \pm SE, n=8. * $p < 0.05$, compared with control; and ** $p < 0.05$, compared with HFD.

The HFD + LO group revealed a considerable decline in fasting serum glucose, insulin level, and HOMA-IR in comparison with the HFD group. In contrast, the HFD + LO group showed significant higher fasting serum glucose, insulin level, and HOMA-IR than the control group.

There was a marked elevation in the level of serum adiponectin in rats fed HFD supplemented with linseed oil compared to the HFD group. While the linseed oil supplemented rats exhibited a significant decrease in serum adiponectin level when compared with control rats.

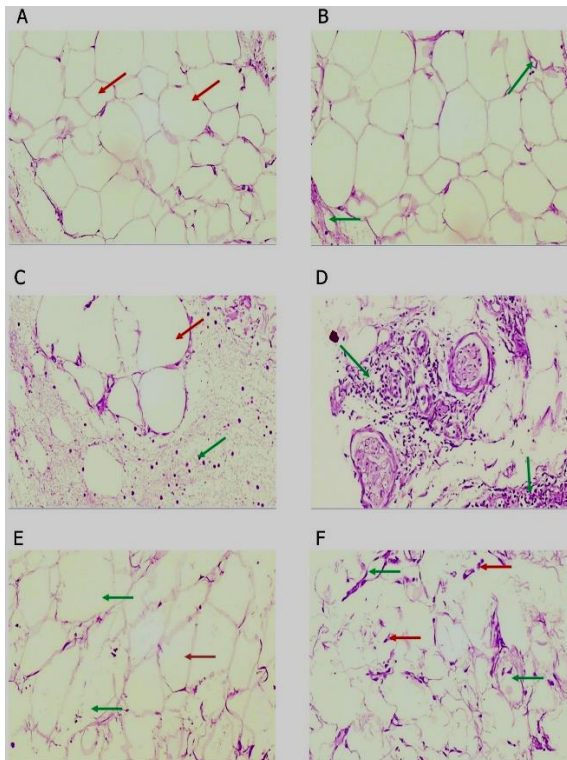
3.3. Effect of linseed oil on adipose tissue histopathology:

To prove the above-mentioned findings, the adipose tissue histological changes that occurred after linseed oil supplementation to the HFD were observed. As considered to the sections of the adipose tissue in photomicrograph. 1 and 2. The control group showed adipocytes of average size are divided by fine fibrous septa containing small blood arteries that are free of congestion or inflammation. On the other hand, the HFD group has massive adipocytes surrounded by infiltration with heavy inflammatory cells, primarily histocytes, and enormous dilated congested blood vessels. Nevertheless, the HFD + LO group elucidated a slight decrease in size of adipose tissue volume with large-sized adipocytes admixed with average-sized adipocytes, moderate dilated blood vessels with mild chronic inflammatory cellular infiltrate, and moderate dilated congested blood vessels.

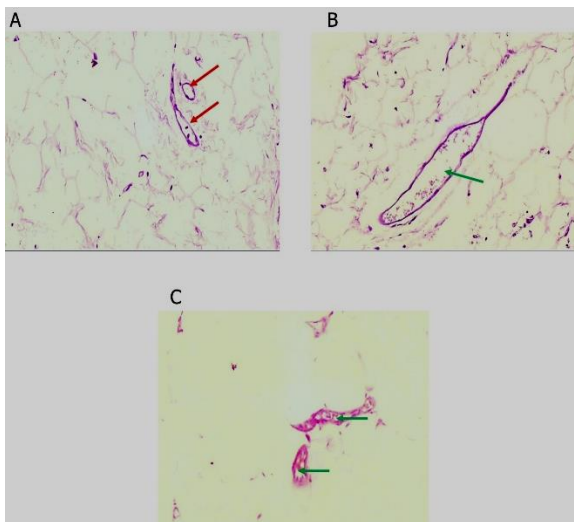
3.4. Effects of linseed oil on mRNA expression of adipose tissue genes:

Fig. 3A revealed that rats were fed HFD significantly increase the RBP4 mRNA level in the adipose tissue when compared to control rats. Whereas linseed oil supplementation substantially reduced the elevation. Also, the linseed oil supplemented group showed a substantial reduction in the mRNA expression level of RBP4 compared with the control group.

In adipose tissue, GLUT4 mRNA expression level was markedly reduced in the HFD group compared to the control group. This reduction was substantially attenuated by supplementation of linseed oil with an HFD diet. On the other hand, the expression of GLUT4 was remarkably decreased in the adipose tissue of the HFD + LO rats compared with control rats (Fig. 3B).



Photomicrograph 1. Histological observations of adipose tissue were stained by H&E and were observed under a microscope ($\times 100$). (A) & (B) sections from the control group. (A) showed the average size of adipocytes separated by fine fibrous septa [red arrows]. (B) showed the average size of adipocytes separated by fine fibrous septa containing small-sized blood vessels without congestion or inflammation [green arrows]. (C) & (D) sections from obese rats fed HFD (HFD group). (C) showed a large size of adipocytes [red arrow] surrounded by inflammatory cells mainly macrophages [green arrow]. (D) showed infiltration with heavy inflammatory cells mainly histocytes (macrophages) [green arrows]. (E) & (F) sections from linseed oil supplemented group (HFD + LO). (E) showed a slight decrease in the size of adipose tissue volume with large-sized adipocytes [green arrows] admixed with average-sized adipocytes [red arrow]. (F) showed moderately dilated blood vessels [green arrows] with mild chronic inflammatory cellular infiltrate [red arrows] (H&E X 100).



Photomicrograph 2. Histological observations of adipose tissue were stained by H&E and were observed under a microscope ($\times 400$). (A) Higher magnification of the adipose tissue of the control group showed average-sized blood vessels without congestion [red arrows]. (B) Higher magnification of HFD group adipose tissue showed large dilated congested blood vessels [green arrow]. (C) Higher magnification of adipose tissue of HFD + LO group showed moderate dilated congested blood vessels [green arrows] (H&E X 400).

4. DISCUSSION

In this study, the HFD group displayed significantly increased body, heart, liver, kidney, and spleen weights compared to the control group. This result is congruous with a previous study (Bashir et al., 2019).

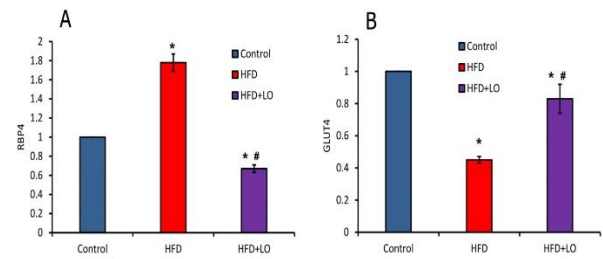


Figure 3. Effect of linseed oil supplementation on the mRNA expression level of (A) RBP4 (B) GLUT4 in adipose tissue of obesity induced rats. The mRNA expression level of RBP4 and GLUT4 were examined by RT-PCR. The relative expression of RBP4 and GLUT4 were normalized to GAPDH. Data are expressed as the mean \pm SE, n=8. * $p < 0.05$, compared with control; and # $p < 0.05$, compared with HFD

Hill, Melanson, and Wyatt, (2000) attributed this rise in body weight to fat's high energy density, which delivers 9 kcal per gram compared to only 4 kcal for carbohydrates and protein. As a result, increasing fat intake can lead to increased energy consumption, energy density, and palatability. The elevation in body weight could also occur as a result of hyperphagia and subsequently high caloric intake stimulated by adipocyte-derived leptin hormone secretion (Dodd *et al.*, 2015). Our data demonstrated that rats were fed HFD with linseed oil supplementation showed a considerable decrease in body, heart, liver, kidney, and spleen weights in comparison to the HFD group, but they were still greater than that of the control group. These results are supported by the study of Bashir et al., (2019) reported that linseed oil supplementation significantly reduced body weight. and Vijaimohan et al., (2006) revealed that linseed oil administration reduces liver weight in Rats fed the HFD in comparison with control rats. Vijaimohan et al., (2006) proposed that the hypolipidemic and antioxidant activities of linseed oil are accountable for its favorable benefits on body weight gain. furthermore, Baranowski *et al.*, (2012) established that the impact of dietary linseed oil on the bodyweight is assigned to its content of α -linolenic acid (ALA) which decreases the adipocyte hypertrophy, protein levels of inflammatory biomarkers, monocyte chemoattractant protein-1 (MCP-1), TNF- α and T-cell infiltration in adipose tissue.

Contrary to our result, Shafie et al., (2019) found that whole linseed, defatted linseed, and SDG treatment caused a non-significant change in the body and liver weights compared to HFD rats. Also, whole linseed treated rats had higher heart weights compared to HFD rats, whereas SDG and defatted linseed treated rats had similar heart weights to HFD rats.

Concerning lipid profile, HFD consumption manifested a significant elevation in serum TC, TG, HDL-C, LDL-C, and VLDL-C in rats when compared with rats receiving a control diet. It has been shown by other investigators that the levels of TC, TG, LDL, and VLDL were substantially elevated in HFD rats in comparison to controls (Han *et al.*, 2018). An important finding in this investigation is that linseed oil substantially reduced the HFD-induced rise in TC, TG, LDL-C, and VLDL-C levels in comparison with the HFD group. Although linseed oil caused a remarkable reduction in these parameters, it was considerably higher than that of the control group. This implies that the linseed oil has an ameliorative effect on the alterations of a lipid profile that occurs due to HFD consumption. These results are congruous with those obtained by Han *et al.*, (2018). Likewise, in this investigation there is a significant elevation of HDL-C level in serum was observed by the effect of supplementation of linseed oil in comparison with that fed on HFD and control diet. Han *et al.*, (2018)

contradict this result in which there is no significant change in HDL levels if compared to HFD rats. The beneficial effect of linseed oil on serum lipid profile attributed to linseed oil contains omega-3 fatty acids, which contribute to the promotion of cholesterol excretion via bile, depleting the hepatic cholesterol pool and enhancing free cholesterol synthesis. Furthermore, ALA-rich diets reduce fat storage in the liver because the acid promotes the β -oxidation of fatty acids and suppresses their synthesis (Murase, Aoki and Tokimitsu, 2005). Linseed oil reduces triglycerides through regulating the peroxisome proliferative-activated receptor (PPAR) and sterol regulatory element-binding protein-1 (SREBP-1), which regulate hepatic fatty acid catabolism and synthesis, respectively (Han *et al.*, 2015).

In the current research, HFD fed rats manifested substantial upraise in serum glucose, insulin levels, and HOMA-IR as compared to control rats. These results coincide with (Yu *et al.*, 2018). Intriguingly, we observed that linseed oil with HFD significantly decrease glucose, insulin levels, and HOMA-IR when compared with HFD. These findings are in the same vein as the previous study by Yu *et al.*, (2018). In contrast, the HFD + LO group showed higher fasting serum glucose insulin levels, and HOMA-IR when compared to the control group. This indicated that linseed oil showed a significant enhancer effect on insulin resistance.

Subsequently, our data explicated that the adiponectin level in serum was observably lower in the HFD group than in the control group. This result is consistent with the study by (Sowers, 2008). The obtained result in our research showed that there was an observable amelioration in the serum adiponectin level in the rats that fed HFD supplemented with linseed oil compared to the HFD fed rats. This result complies with the study by Baranowski *et al.*, (2012). Linseed's ability to induce adiponectin may be due to its high ALA content. ALA plays an important role in raising adiponectin secretion via activating the transcription factor PPAR γ (Kratz *et al.*, 2008). PPAR is a crucial transcription factor that controls the expressions of adiponectin, leptin, and glucose transporter type 4 (GLUT4) in addition to regulating adipogenesis (Prasad, 1999). Furthermore, SDG in linseeds may act as a PPAR agonist and regulate adiponectin through an increasing the PPAR γ DNA binding activity in adipocytes (Fukumitsu *et al.*, 2008). So, linseed may be a crucial component that alters the adipose tissue metabolic process, resulting in less visceral fat accumulation (Ahmadniay motlagh *et al.*, 2021)

The current histopathological observations demonstrated that the HFD group has massive adipocytes surrounded by infiltration with heavy inflammatory cells, primarily histocytes, and enormous dilated congested blood vessels. Our observations are in agreement with the former study (Bashir, Ali and Khan, 2015). Interestingly, we observed that the HFD + LO group elucidate a decrease in the size of adipose tissue volume with large-sized adipocytes mixed with average-sized adipocytes, moderate dilated blood vessels with mild chronic inflammatory cellular infiltrate and moderate dilated congested blood vessels. This indicates that the linseed oil-induced an enhancement in the physiology of adipose tissue. This correlated with prior studies (Bashir, Ali and Khan, 2015; Bashir *et al.*, 2019). The improvements in obesity indicators could be explained by these findings. Because the larger adipocyte of obese rats causes a change in the profile of the pro and anti-inflammatory adipokines. This means shifting the balance to more pro-inflammatory adipokines like RBP4 and less anti-inflammatory adipokines like adiponectin.

Intriguingly, our result showed that the RBP4 mRNA expression level in adipose tissue was considerably increased in rats fed HFD. This result is confirmed by the previous study (Zhu *et al.*, 2015). On the other side, the GLUT4 mRNA expression level in adipose tissue was substantially reduced by HFD. This result keeps in harmony with Flachs *et al.*, (2014). Yang *et al.*, (2005) discovered that retinol-binding protein 4 (RBP4) is an adipokine and the visceral adipose tissue is considered as the main source of it. RBP4 expression and secretion in adipose tissue were found to be intimately linked to glucose uptake and insulin sensitivity. The expression of glucose transporter 4 (Glut4) is reduced in adipocytes in an obese or diabetic condition, and this decrease is accompanied by an upraise in RBP4 expression and secretion into the blood (Graham *et al.*, 2006). This explained our results of RBP4 and GLUT4 mRNA expression level of adipose tissue in obese rats. The elevation provokes disruption of insulin signaling in skeletal muscle and promotes glucose synthesis in the liver. These alterations result in a high blood glucose level (Graham *et al.*, 2006). Subsequently, adipocyte Glut4-RBP4 system dysregulation is intrinsically related to insulin resistance and type 2 diabetes mellitus., as well as insulin-resistant conditions, such as nonalcoholic fatty liver disease and metabolic syndrome (Lin *et al.*, 2013). The effect of linseed oil on RBP4 expression in adipose tissue has not been previously studied. Interestingly, our study revealed that linseed oil supplementation with a high-fat diet significantly reduced the elevation. Also, linseed oil supplemented rats manifested a considerable reduction in the mRNA expression level of RBP4 when compared with the control group. Subsequently, we hypothesized that the linseed oil would induce the expression of GLUT4 in adipose tissue according to Flachs *et al.*, (2014) reported that in rodents, both GLUT4 expression and glucose transport are inhibited by a high-fat diet in parallel with the induction of IR, while admixing of EPA and DHA, which are generated from ALA metabolism to the diet have protective effects. In line with our presumption, our study revealed a significant finding that the reduction of GLUT4 expression caused by HFD was substantially attenuated by supplementation of linseed oil with an HFD diet.

The impact of linseed oil in RBP4 could be attributed to the alleviation effect of linseed oil on visceral adipose tissue (Fukumitsu *et al.*, 2008). In the same aspect former study Baranowski *et al.*, (2012) showed that dietary ALA-rich linseed oil decreased the size of adipocytes (hypertrophy) by 17% in obese rats. Also, Bashir *et al.*, (2019) observed a profound decrease in the epididymal and retroperitoneal fat pad weight of groups fed HFD with linseed oil in comparison to the HFD group. Our histopathological examination also confirms these results. These results signify that linseed oil has an anti-inflammatory effect, which is clarified by the ameliorative effect of linseed oil on the level of adiponectin in serum and on the expression of RBP4 in adipose tissue.

5. CONCLUSION

In conclusion, linseed oil supplementation alleviates the metabolic disorders accompanying obesity such as hyperlipidemia, insulin resistance, elevated pro-inflammatory cytokines, and low anti-inflammatory cytokines.

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