

Full Length Research Paper

Ameliorative effect of grape seed extract on metabolic disorders caused by high fat diet induced obesity in rats by reversing the increase in hepatic miR-33a and miR-122

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MiR-33a and miR-122 are major regulators of lipid metabolism in the liver and their deregulation has been linked to the development of metabolic diseases such as obesity and metabolic syndrome. The aim of this study was to evaluate whether the level of miR-33a and miR-122 in rat liver correlate with obesity and potential anti-obesity effect of grape seed extract (GSE) and calorie restricted diet with special emphasis on dyslipidemia, oxidative stress and inflammation. Rats received high fat diet (HFD) for four months to induce obesity. Animals which had $\geq 30\%$ increase in body weight were selected in this study. Obese rats were divided into 4 groups (n=12/each) and treated for 8 weeks with caloric restriction, GSE (30 mg/kg daily orally) alone or in combined form. Obese rats developed increased body weight and up-regulation of miR-33a and miR-122 in the liver. Also obesity provoked dyslipidemia, oxidative stress and inflammatory status. Importantly, GSE alleviated all deleterious effects of HFD especially when administered with calorie restricted diet. They counteracted the increase of these two miRNAs with improvement in dyslipidemia, oxidative and inflammatory processes. The results reported suggested that beneficial metabolic effects of GSE in combination with dietary treatment could be useful to treat obesity and metabolic disorders.

Key words: Obesity, GSE, dyslipidemia, oxidative stress, hepatic miRNAs

INTRODUCTION

Obesity is a public health concern characterized by excessive fat deposition into adipocytes and non-adipose tissues, which is accompanied by a cluster of chronic metabolic disorders, including cardiovascular diseases,

type 2 diabetes, steatohepatitis and dyslipidemia. Obesity and metabolic disorders are also linked to an overt oxidative stress and chronic inflammatory status. Oxidative stress along with a decline in antioxidant

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defenses cause an irreversible damage to macromolecules (Levine and Stadtman, 2000) and a disruption in redox signaling mechanisms (Kamata and Hirata, 1999). High-fat feeding has commonly been used to induce visceral obesity in rodents because of the similar pathogenesis with abdominal obesity found in human (Katagiri et al., 2007).

Dyslipidemia is a known complication of obesity (Sowers, 2003). Obese children and adults particularly those with a central or abdominal distribution of fat, have elevated concentration of serum triacylglycerol (TAG) surrogate measure of very low density lipoprotein cholesterol (VLDLc) and low concentration of high density lipoprotein cholesterol (HDLc) (Freedman et al., 2002). Obesity is also associated with higher levels of total cholesterol (Tc) and low density lipoprotein cholesterol (LDLc) (Knopp et al., 2008). Obesity enhances oxidative stress in young and old populations as shown by elevations in lipid peroxidation or protein oxidation. Lipid peroxidation is associated with several indices of adiposity and low systemic antioxidant defense that is, antioxidant enzymes, glutathione (GSH) (Vincent et al., 2007). It plays a role in the initiation of inflammation and development of insulin resistance (Tilg and Moschen, 2008). Insulin resistance (IR), hyperinsulinemia and hyperglycemia are linked with obesity (Sowers, 2003). Obesity-induced inflammation results in increased infiltration of macrophages and release of cytokines like tumor necrosis factor alpha (TNF α), interleukin-6 (IL-6) and interleukin-1-beta (IL-1 β) and contributes significantly to insulin resistance (Larsen et al., 2007).

MiRNAs are known to modulate more than 60% of human transcripts and thus play important regulatory roles in a variety of biological processes and are implicated in almost all metabolic pathways (Friedman et al., 2009). Moreover, there is much evidence that the deregulation of miRNAs is related to the development of chronic diseases (Rottiers and Naar, 2012). Specifically, miR-33 and miR-122 are known as major regulators of lipid metabolism in the liver, and their deregulation may contribute to the development of metabolic diseases such as obesity and metabolic syndrome (Rottiers and Naar, 2012; Ramirez et al., 2011). MiR-122 plays a critical role in liver homeostasis by regulating genes with key roles in the synthesis of triglycerides (TGs) and fatty acids (FAs), such as FA synthase (FAS) and sterol regulatory element-binding protein 1C (SREBP1C), as well as genes that regulate FA β -oxidation (Tsai et al., 2012; Hus et al., 2012).

Additionally, miR-33a plays an important role in the regulation of cholesterol homeostasis in the liver, regulating the ATP-binding cassette transporters (ABC transporters) ABCA1 and ABCG1 in addition to its role in FA β -oxidation by targeting the carnitine palmitoyltransferase 1a (CPT1a) (Moor et al., 2011). Since current medical treatments fails to stop the progress of metabolic disorders, polyphenol-rich grape products are being widely investigated as an additional strategy to

combat obesity (Chuang and McIntosh, 2011). The grape seed skin extract (GSSE) exerts numerous biological activities and health-promoting properties such as antioxidant (Belvir'nli et al., 2012), lipid lowering (Quesada et al., 2012), anti-tumor (Nandakumar et al., 2008) and anti-obesity effects (Ohyama et al., 2011) by inhibiting lipid absorption from the intestine which has been shown to occur partly via inhibition of lipase (Moreno et al., 2003).

In the present study, we evaluate whether the level of miR-33a and miR-122 in rat liver correlate with obesity and potential anti-obesity effect of grape seed extract with or without calorie restricted diet with special emphasis on dyslipidemia, oxidative stress and inflammation.

MATERIALS AND METHODS

Chemicals and drugs

Gervital (grape seed extract: GSE) was purchased from Arab Co. for Pharmaceuticals and Medicinal Plants (MEPACO-MEDIFOOD), Sharkeya-Egypt. All other chemicals used in this study were of analytical grade obtained from Sigma Aldrich, USA, unless otherwise noted.

Animals and experimental design

Male Wistar rats (n= 100, 160 \pm 15 g) were purchased from Egyptian Organization for Biologic Products and Vaccines (Cairo, Egypt). All experimental protocols were approved by the Animal Experimental Ethics Committee of Faculty of Pharmacy, Zagazig University. Every effort was made to minimize the number of animal used and their suffering. Rats were housed in stainless steel rodent cages at room temperature (25 \pm 2°C) and with 12 h dark/light cycle. Animals were fed rodent chow and allowed free access to drinking water. One week after acclimatization, eighty eight were switched from rodent chow to high fat diet (25% total fat (including 11% unsaturated fat, 44% carbohydrate, 18% protein and 13% fiber and other ingredients) for four months to induce obesity (Alzoubi et al., 2009). The animals which had \geq 30% increase in body weight were selected in this study. The obese rats were trained to lick suspension of gum acacia in distilled water (1 ml) which was used as the vehicle and randomly divided into four groups: Obese control (OC; n=12, kept on normal chow diet), calorie restricted group (CR; n=12), 25% food restriction of commercial chow (Luvizotto et al., 2010), gervital group (Ger; n=12 receiving GSE 30 mg/kg daily orally dissolved in distilled water using gum acacia as suspending agent kept in normal chow diet) and calorie restricted + gervital group (CR + Ger; n=12) for 8 weeks. In addition to normal control (NC, n=12).

Biochemical studies

At the end of experimental period, body weight was determined for all groups, then rats were anesthetized with urethane (1.3 g/kg) and blood samples were collected from orbital sinus of rats according to (Sorg and Buckner, 1964) then centrifuged at 3000 rpm for 15 min. Serum was collected, divided into aliquots and stored at -20°C for the determination of total cholesterol (Tc), triacylglycerol (TAG), high density lipoprotein (HDLc) using commercially available kits spinreact sant Esteve de Bas Spain. Low density lipoprotein (LDLc)

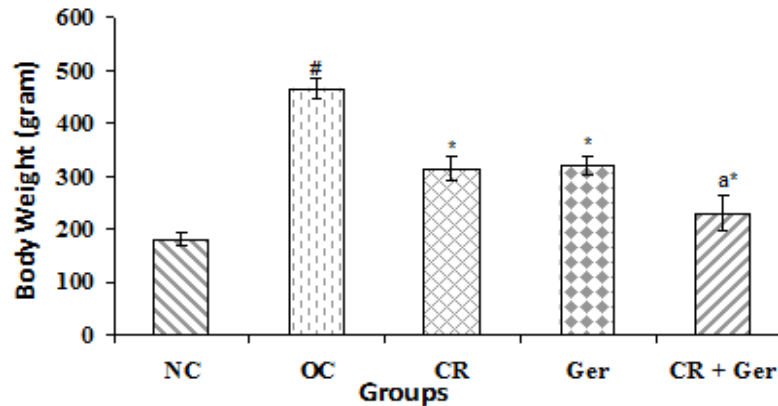


Figure 1. Effect of CR, Ger and their combination on body weight. Values are expressed as mean \pm SD (n=12) ($P < 0.05$). #Significantly different from NC group. *Significantly different from OC. a*Significantly different from CR. NC: Normal control, OC: Obese control, CR : Calorie restricted, Ger: Gervital

was calculated according to Friedewald formula (Friedewald et al., 1972):

LDLc (mg/dl): Tc-(HDLc + TAG/5).

Serum glucose level was done using kit according to the method of Trinder (Trinder 1969), serum insulin was estimated by radioimmunoassay technique using insulin kit purchased from Siemens Medical Solutions Diagnostics, Los Angeles, USA according to method of Bennett (Bennett, 1983). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using serum glucose and insulin level according to Matthews et al. (1985) $HOMA-IR = (\text{glucose} \times \text{insulin})/405$. Serum TNF α was assayed according to Chen et al. (1998) by ELISA using rat TNF α kits (Ray Biotech, USA). Serum andiponectin was measured using enzyme assay (ELISA) kits (Invitrogen) according to the manufacturer's instruction.

After blood collection, rats were euthanized by CO₂ asphyxiation, livers were removed, washed with 0.9% NaCl. Part of harvested organs was quickly frozen in liquid nitrogen (-170°C) for analysis of superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), total antioxidant capacity (TAC) using spin react kits Spain. Malondialdehyde (MDA) was measured spectrophotometrically by modified method of Buege and Aust (Buege and Aust, 1978). Reverse transcription-polymerase chain reaction (PCR) was used to analyze the miR-33a and miR-122 gene expression. To analyze the expression of each miRNA, reverse transcription was performed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster city, USA). miRNA-Specific reverse-transcription primers were provided by TaqMan MicroRNA Assay (Applied Biosystems, Foster City, USA). For the reverse transcription, a Biometra thermal cycler was used. The reaction was performed at 42°C for 30 min and 95°C for 5 min. A total of 1.33 ml of this diluted cDNA was used in a subsequent quantitative reverse transcriptase polymerase chain reaction (RT-PCR) amplification using TaqMan Universal PCR master mix (Applied Biosystems, Foster City, USA) and the specific probe provided in the TaqMan MicroRNA Assay (Applied Biosystems). Specific Taqman probes were as follows: microRNA-122 (miR-122: hsa-mir-122), 5'-UGGAGUGUGACAAUGGUGUUUG-3', and microRNA-33 (miR-33: hsa-mir-33), 5'-GUGCAUUGUAGUUGCAUUG-3'. The results were normalized with U6 small nuclear RNA (U6 snRNA), 5'-TGCTCGCTTCGGCAGCACATATACTAAAATTGGAACGATACAA GAAGATTAGCATGGCCCCTGCGCAAGGATGACACGCAAATTC

GTGAAGCGTTCATATTTT-3', that was used as an endogenous control. Amplification was performed using the StepOne Plus Real-Time PCR system (Applied Biosystems, Foster City, USA) at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The fold change in the miRNA level was calculated by the log 2 scale of the equation $2Ct$, where $Ct = Ct \text{ miRNA} - Ct \text{ U6}$ and $Ct = Ct$ treated samples Ct untreated controls.

Statistical analysis

All data were measured as mean \pm standard deviation. Results were analyzed by analysis of variance ((ANOVA); 1-way) followed by Tukey Post Hoc test, $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS (version 16 SPSS Inc. Chicago, USA). However the correlations between the studied parameters were assessed using the person's correlation coefficient (r).

RESULTS

Body weight

Body weight increased significantly in obese group by 158.2% after four months high fat diet compared to NC group while significant decreases were observed after 8 weeks in the CR, Ger either individually or in combination by 32, 31 and 50.6%, respectively compared to OC animals and CR + Ger group showed a significant reduction by 27% than CR only ($P < 0.05$) (Figure 1).

Biochemical evaluation

Serum parameters

Serum lipid profile: Obese rats demonstrated significant increase in Tc, TAG, LDLc and atherogenic index by 61.9, 44.6, 161 and 27.6%, respectively associated with

Table 1. Effect of Cr, Ger either individually or in combination in lipid profile in obese rats.

Concentration (mg/dl)	Normal	Obese	CR	Ger	CR + Ger
Tc	84 ± 4.5	136.5±8.1 [#]	109±11.1*	107±10.5*	87.9±6.9 ^{*a}
TAG	62.2 ± 4.5	112±10.2 [#]	88.1±8*	70.9±3.1*	64.1±9.2 ^{*a}
LDLc	34.5 ± 2	88.9 ±1.8 [#]	55.4±4*	57±9.5*	37.2±2 ^{*a}
Atherogenic index	0.93 ± 0.1	3.5±0.4 [#]	1.5±0.17*	1.6±0.28*	1.0±0.07 ^{*a}
HDLc	37.1 ± 4.3	25.2±3.9 [#]	36±6.1*	35.9±6.1*	37.5±3.9 [*]

Values are expressed as mean ± SD (n=12) (P<0.01). [#]Significantly different from NC group. ^{*}Significantly different from OC group. ^aSignificantly different from Cr group

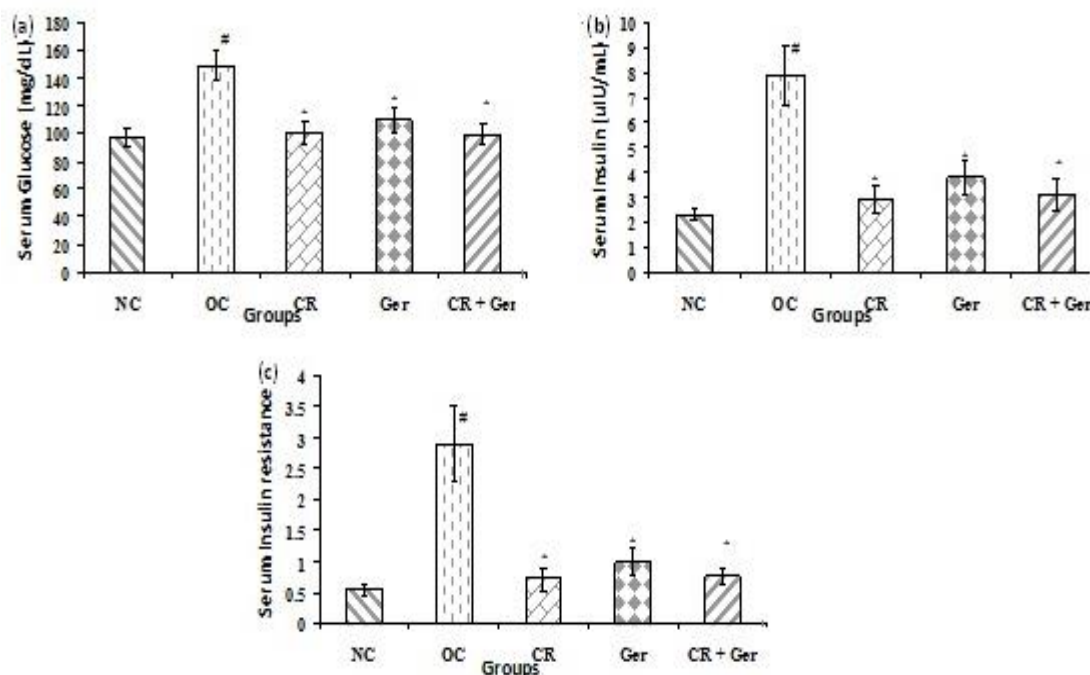


Figure 2. Effect of CR, Ger and their combination on serum glucose, insulin and insulin resistance (HOMA) (a,b,c). Values are expressed as mean ± SD (n=12) (P<0.05). [#]Significantly different from NC group. ^{*}Significantly different from OC. ^aSignificantly different from CR.

significant decrease in HDL by 32.4% in comparison with NC group. These alterations in lipid profile were significantly ameliorated in all treated groups as compared to OC Rats. Co-administration of gervital with caloric restriction significantly lowered Tc, TAG, LDLc and atherogenic index by 20.1, 27.2, 32.7 and 33%, respectively compared to calorie restricted group (P<0.05). The HDLc showed non-significant change (Table 1).

Serum glucose, insulin level: Obese rats had a significant increase in serum glucose level by 52.2% and as compared to NC group. This elevation in glucose level was accompanied by a significant increase in insulin level and insulin resistance by 243.5 and 427%, respectively.

Obese rats received CR or Ger either individually and in combined form showed a remarkable improvement in these parameters in comparison with OC group. Co-administration of gervital with calorie restricted diet demonstrated non significant change in comparison to CR group (P<0.05) (Figure 2).

Serum adiponectin: Obese rats demonstrated significant (P<0.05) decrease in serum adiponectin by 45.5% in comparison with normal group. Treatment with calorie restricted diet or gervital either individually and in combined form significantly increased adiponectin level by 27.7, 44.4 and 42.3%, respectively as compared to obese rats. Combination treatment showed remarkable increase in its level by 34.8% in comparison with CR group

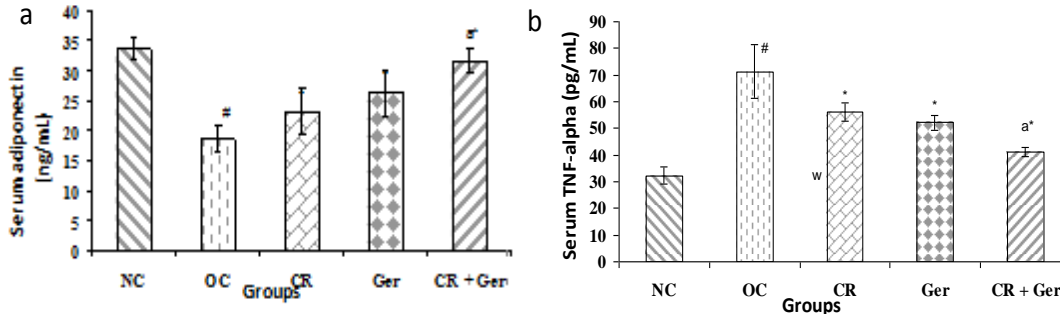


Figure 3. Effect of CR, Ger and their combination on serum adiponectin and TNF α (a,b). Values are expressed as mean \pm SD (n=12) (P<0.05). #Significantly different from NC group. *Significantly different from OC. ^Significantly different from CR

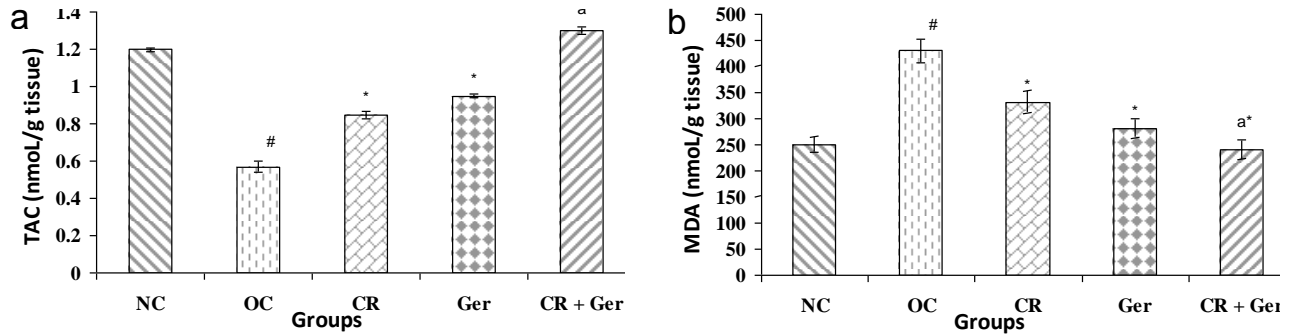


Figure 4. Effect of CR, Ger and their combination on hepatic TAC and MDA (a,b). Values are expressed as means \pm SD (n=12) (P<0.005). #Significantly different from NC group. *Significantly different from OC. ^Significantly different from CR.

(P<0.05) (Figure 3).

Serum TNF α : The serum TNF α level was significantly higher in obese rats by 121% than normal group (P<0.05). After treatment with calorie restricted diet and gervital, this cytokine was lowered especially in CR plus gervital group (Figure 3).

Tissue parameters

Hepatic TAC and MDA: The result of present study have demonstrated that obese rats showed a significant decrease in hepatic TAC capacity by 52.5% compared to normal group. This decrease in TAC is accompanied by increase in hepatic MDA by 72%. Caloric restriction or gervital administration either individually and in combined form notably inhibited the elevation in of hepatic MDA by 23.2, 34.9 and 44.2%, respectively and exert significant enhancement on hepatic TAC by 49.1, 66.7 and 128% in comparison with obese rats. Dietary regimens and drug therapy significantly increase TAC by 52.9% and decrease MDA by 27.3% in comparison with CR group

(P<0.05) (Figure 4).

Hepatic antioxidant enzyme activity: Key antioxidant enzymes including SOD and CAT were measured. Obese rats showed significant decrease in all antioxidant enzyme activities by 53.9 and 30%, respectively when compared to the normal control. However, calorie restricted diet or gervital either individually and in combined form significantly (P<0.05) increased the antioxidant enzyme activities compared to that of obese control group. Co-administration of gervital with calorie restricted diet significantly elevates antioxidant enzyme activities by 36.4 and 25.3% compared to CR group (Figure 5).

Hepatic GSH: Liver GSH level were decreased significantly in obese rats by 44.4% in comparison to normal control (P<0.05). Calorie restricted diet or gervital therapy either individually or in combination significantly restored GSH levels near normal values compared to obese rats. However calorie restricted diet in combination with gervital therapy induced a significant elevation in GSH level by 21.9% in comparison with CR group (Figure 6).

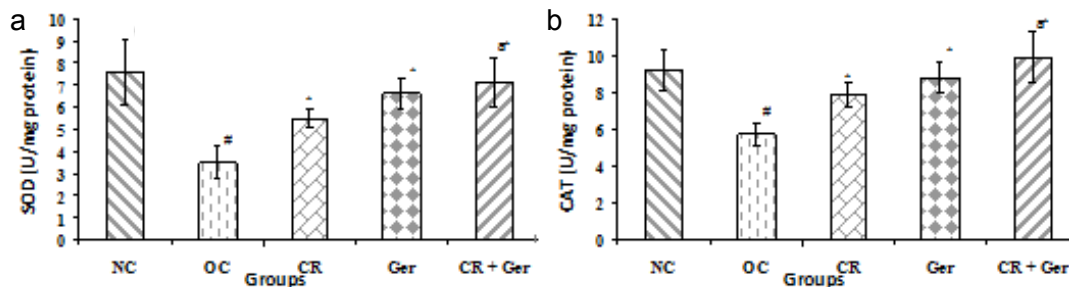


Figure 5. Effect of CR, Ger and their combination on hepatic antioxidant enzyme activity (a,b). Values are expressed as means \pm SD (n=12) (P<0.05). #Significantly different from NC group. *Significantly different from OC. ^aSignificantly different from CR.

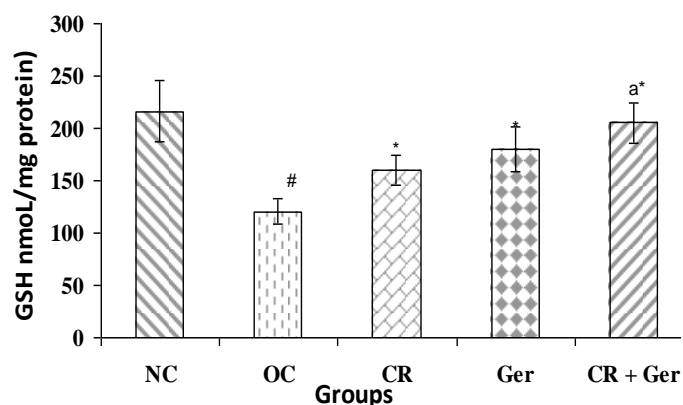


Figure 6. Effect of CR, Ger and their combination on hepatic GSH. Values are expressed as means \pm SD (n=12) (P<0.05). #Significantly different from NC group. *Significantly different from OC. ^aSignificantly different from CR.

Hepatic miR-33a and miR-122: High fat diet significantly increase miR-33 and miR-122 gene expression in liver tissue by 358 and 267%, respectively in comparison to normal rats (P<0.05). Treatment with caloric restriction or gervital either individually or in combination significantly reversed the increase in miR-33a and miR-122 leading to reduction by (36.4, 40.9, and 75%) and (40, 44 and 70%), respectively in their level compared to obese rats. However when gervital and caloric restriction were administered in combination, the reduction was greater by 60.7 and 51.3%, respectively than caloric restriction alone (P<0.05) (Figure 7 to 9).

Correlation study

Using the combined results from all groups, we illustrated that both hepatic miR-33a and miR-122 were positively correlated with all lipid fractions (except HDLc), tumor necrosis factor alpha and adiponectin. On the other hand, they were negatively correlated with hepatic antioxidant parameters but positively correlated with MDA.

DISCUSSION

The current work handled the effect of high fat diet in the induction of obesity. The latter was manifested by a marked increase in body weight. Obese rats developed dyslipidemia as manifested by a significant increase in Tc, TAG, LDLc, atherogenic index and reduction on HDLc accompanied by significant elevation in blood glucose level and remarkable increase in insulin intolerance. With regard to the liver, HFD also provoked a clear oxidative stress status as evidenced by increased MDA, decreased reducing power (GSH and TAC) and inhibition of antioxidant enzyme activities as CAT and SOD and inflammation as shown by elevation in hepatic TNF α . Interestingly, in association with dyslipidemia, inflammation and oxidative stress, the level of miR-33a and miR-122 were upregulated in obese rats.

Previous study, Kappes and Loffler (2000) suggested that there is increasing evidence that obesity impairs adipocyte function and secretion of adipocytokines (adiponectin and TNF- α). The reduction in adiponectin level is due to increasing TNF- α which inhibits adiponectin expression in adipose tissue (Li et al., 2009). Moreover circulating adiponectin is inversely correlate with plasma TNF- α (Bruun et al., 2003). Reduced levels of adiponectin contribute to development of insulin resistance. Adiponectin is negatively correlate with insulin resistance (Hotta et al 2000). Havel (2002) reported that adiponectin can reduce glucose level so this hypoglycemic effect is associated with increase insulin sensitivity. MiRNAs have been described as regulators of gene expression and the deregulation of several miRNAs that are related to chronic diseases has been reported (Rottiers and Naar 2012).

Specifically, miR-33a and miR-122 play key roles in lipid metabolism. It is well known that these two miRNAs are involved in cholesterol and TAG metabolism (Krutzfeldt et al 2005 and Esau et al 2006). So deregulation of miR-33a and miR-122 in obese rats has been related to the development of dyslipidemia. Inhibition of miRNA-122 in mice results in a significant

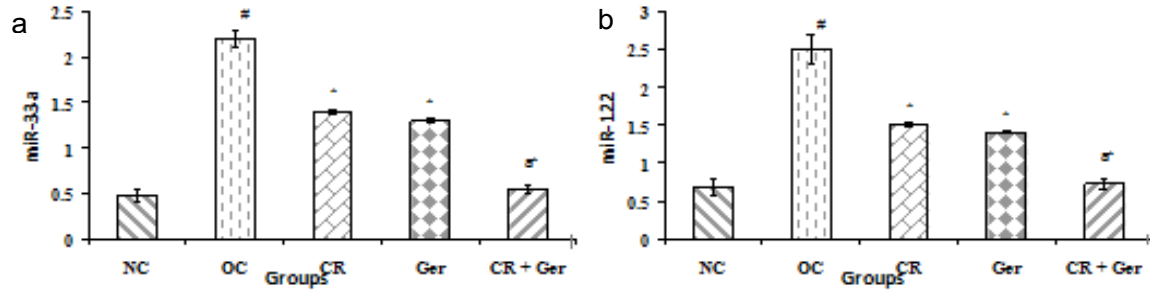


Figure 7. Effect of CR, Ger and their combination on hepatic miR-33a and miR-122 (a,b). Values are expressed as means ± SD (n=12) (P<0.05). [#]Significantly different from NC group. ^aSignificantly different from OC. ^bSignificantly different from CR.

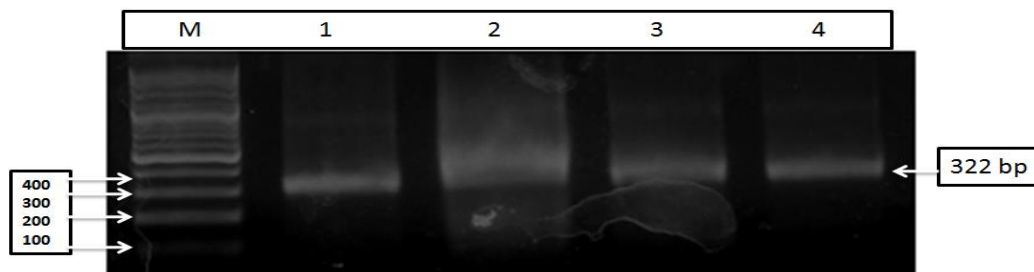


Figure 8. Agarose gel electrophoresis showed PCR product of mir RNA 33a gene with 322 bp size in RNA extract. Lane M: DNA ladder (100, 200, 300, 400 bp.....etc) .Lane 1-4: PCR products of positive samples in all studied groups

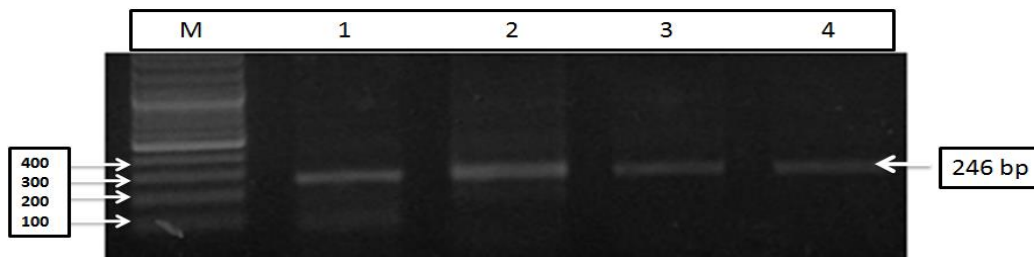


Figure 9. Agarose gel electrophoresis showed PCR product of mir RNA 122 gene with 246 bp size in RNA extract. Lane M: DNA ladder (100, 200, 300, 400 bp.....etc) .Lane 1-4: PCR products of positive samples in all studied group.

Table 2. Correlation between hepatic miR-33a and miR-122 with serum lipid fractions, tumor necrosis factor alpha (TNF) adiponectin.

Serum parameter	MiR-33 a	miR- 122 a
TC	r=0.82	r=0.72
TAG	r=0.72	r=0.81
LDL	r=0.85	r=0.69
HDL	r=-0.59	r= -0.79
LDL / HDL	r=0.69	r=0.62
TNF	r=0.81	r=0.88
adipotectin	r=0.71	r=0.79

Significant at P<0.0001

reduction in plasma cholesterol and TAG levels, Mechanistically, Tsai and colleagues (2012) found that the absence of miR-122 results in a significant reduction of microsomal transfer protein (MTTP) expression, thereby decreasing very low density lipoprotein (VLDL) secretion from liver Furthermore, the silencing of miR-33a by knockout or antisense techniques in mice results in an improvement in plasma lipid profile with increasing (HDLc) level (Rayner et al., 2011). These results were confirmed by correlation between miR-33a miR122 with Tc, TAG, LDLc and negative correlation with HDLc (Table 2).

Generally dyslipidemia observed in obesity is mainly

Table 3. Correlation between hepatic miR-33a and miR-122 with hepatic oxidant and antioxidant parameters.

hepatic oxidative and antioxidant parameters	MiR-33 a	miR- 122 a
MDA	r= 0.81	r= 0.62
GSH	r= -0.75	r= -0.77
TAC	r=-0.85	r= -0.81
CAT	r= -0.72	r= -0.59
SOD	r= -0.66	r= -0.69

Significant at $P < 0.0001$

attributed to decreased activity of lipoprotein lipase. The LPL is an insulin sensitive enzyme which demonstrates significant alteration in many diabetics (Adiels et al., 2006). The reduction of LPL activity is sufficient to reduce the clearance of chylomicrons and VLDL in insulin resistance syndrome (Markel et al., 2002). Hosogai et al. (2007) demonstrated that hypoxia in adipose tissue associated with obesity results in endoplasmic reticulum (ER) stress due to accumulation of unfolded proteins in the ER. The ER stress causes down regulation of adiponectin through activation of C/EBP homologous protein (CHOP) that heterodimerize with C/EBP and form inactive complex. It is stated that C/EBP is critical for regulation of adiponectin transcription.

Obesity is linked to an overt oxidative stress along a decline in antioxidant defenses (Kamel et al., 2013) HFD cause reactive oxygen species (ROS) accumulation, an increase in lipoperoxidation and protein carbonylation and decrease in thiols radicals and glutathione. Consequently our data further confirmed that HFD also inhibit SOD, CAT and GPx activity leading to depletion of glutathione and reduction in TAC (Lee et al 2008). Inhibition of miR-33a also decrease the expression of proinflammatory and prooxidant genes including inducible nitric oxide synthase and tumor necrosis factor alpha (Ho et al., 2011). Also inhibition of miR122 decreases the basic leucine zipper transcription factor-1 (BACH-1) and increases heme oxygenase -1 (OH-1) a key cytoprotective enzyme with antioxidant properties repressed by BACH-1 (Shan et al., 2007). These results were confirmed by correlation studies (Table 3)

The current study demonstrated that obese rats treated with CR or GSE exhibited an improvement in lipid profile where Tc, TAG, LDLc and atherogenic index were decrease while HDL was elevated, significant decrease in blood glucose, insulin and IR, an elevation in TAC, CAT, SOD and glutathione and reduction in MDA. Adiponectin is significantly increased while TNF- α is decreased in addition to miR-33a and miR-122 are also elevated compared to obese rats.

Lipid lowering property of CR is possibly due to increase level of adiponectin (Ding et al., 2012) who have proposed that CR have a role in protecting against insulin resistance through lowering adiposity and up-regulation of adiponectin expression. Adiponectin plays major role in

the regulation of glucose, insulin and fatty acids and has anti-obesity effect. It can increase β -oxidation in tissues and causes weight loss and is inversely related with fat mass (Hu et al., 2012). Also adiponectin over expression confers dramatic metabolic improvement that includes decreased TAG, Tc, Fat cell size and increased fat cell number and improved glucose tolerance (Trujillo and Scherer, 2006). The marked decreased in TNF- α level is greatly joined with elevated adiponectin concentration (Cawthorn and Sethi, 2008). The ability of CR to restore antioxidant defense mechanism and elevation of miR-33a and miR-122 may be due to its weight lowering effect.

The current study demonstrated that obese rats treated with gervital exhibited the powerful ability of grape seed extract (GSE) to counteract most of the HFD induced disturbance such as weight gain, dyslipidemia, IR oxidative stress, low grade inflammation and upregulation of miR-33a and miR-122 the major regulators of lipid metabolism in the liver. In line with our results, previous studies have suggested that GSE could be an effective therapeutic agent for obesity. Pajuelo et al. (2012) showed that GSE administration protected against weight gain in wistar rats with obesity induced by high fat diet. Improvement of lipid fraction to be close to normal level after GSE administration indicating its effect in intestinal absorption (Sugiyama et al., 2007).

Our data are fully in line with those of Caimari et al. (2013) who showed the beneficial anti-obesity effect of grape seed procyanidins which increased lipase activity in white adipose tissue in hamsters. Literature survey revealed that GSE exhibit insulinomimetic properties (Adisakwattana et al., 2010) and antihyperglycemic effects (Suwannaphet et al., 2010). These effects may be due to antidiabetic activity of the natural plant phenolic compounds (You et al., 2012). Consequently GSE increases the level of liver enzyme activities of SOD, CAT as well as GSH. Such treatment caused augmentation in serum insulin, hepatic TAC levels accompanied by decrease in serum glucose and liver MDA. The antioxidant activity of GSE may be due to the inhibition of oxidation of plasma lipids. Moreover it is able to scavenge hydroxyl radicals, peroxy radicals superoxide anion radicals (Yilmaz and Toledo, 2004).

When weight gain was reduced with GSE, the capabilities of GSE as an anti-inflammatory was identified

in our study. Therapeutic inhibition of C-reactive protein (CRP) by GSE is considered as a promising new approach to cardioprotective and myocardial infarction or to other inflammatory, infective and tissue damaging conditions characterized by increased CRP level (Pepys et al., 2006). Altogether, our data highlighted the antioxidant and anti-inflammatory role of GSE (Bagchi et al., 2000).

Interestingly, GSE treatment counteracted the overexpression of miR-133 and miR-122 induced by HFD. Our data is in agreement with previous studies (Laura et al., 2013) who stated that the repression of rat liver miR-33a and miR-122 induced by GSE was clearly associated with the improvement in plasma lipid profile, it could be suggested that the modulation of miR-33a could be one of the molecular mechanism used by GSE to improve the plasmatic atherogenic profile that was induced by HFD.

Our study revealed that using a combination between CR and GSE might synergically improve lipid profile except HDLc which showed non significant change in comparison to CR group. We evaluated effect of this combination in blood glucose, insulin and IR. In this case the effect was showed to a similar degree to that found when administering separately and the effect was not additive or synergistic.

In the present study, the tested combination of both CR diet and GSE on oxidative stress markers may reflect the sum of efficiency of either administration alone. Thus acting with more than one mechanism of action including, the reduction of ROS and scavenging of free radicals as well as improvement of the tissue enzymatic and non enzymatic antioxidant activities. When the two treatments were orally administered together the increment of hepatic adiponectin content showed an additive effect in CR-Ger group accompanied with synergic decrease in hepatic TNF- α . Additionally, co-administration repressed the liver expression of miR-33a and miR-122 greater than when the treatments were administered separately. Previously, we have shown that GSE repress miR-33a and miR-122 liver expression in rats treated with an acute dose which also induced postprandial hypolipidemia in normal rats (Baselga et al., 2012).

Conclusion

The level of miR-33a and miR-122 in the liver correlate with metabolic disorders induced by high fat diet. Grape seed extract in combination with caloric restriction is considered a promising therapy for treatment of metabolic disorders associated obesity by down regulation of hepatic miR 33a and miR 122. More studies are necessary to elucidate the exact mechanism by which CR and GSE repress miR-33a and miR-122.

Conflict of interest

The authors have not declared any conflict of interest.

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