Resveratrol attenuates hepatic complications associated with insulin resistance: Implications on hepatic HAIR, LAIR, cell energy and DNA fragmentation

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ABSTRACT

Metabolic syndrome (MetS) is a chronic condition, often related to obesity, improper diet, insulin resistance (IR); and can lead to significant health complications. Resveratrol (RSV) is a naturally occurring polyphenol recently postulated to be a powerful antioxidant, hepatoprotective and a potential anti-hyperglycemic. Our research aimed to investigate some possible mechanisms of action that may contribute to the efficacy of RSV to reverse the IR and hepatic complications associated with MetS experimentally induced in rats using high fat-high fructose (HFHF) diet model. Results revealed that RSV treatment (40 mg/kg p.o) for ten days protected against IR and hepatic insult as demonstrated by reduction in HOMA-IR, hepatic HAIR and LAIR expression, total cholesterol (TC), triglycerides (TG), TNF-α levels, oxidative and nitrosative stresses, serum AST and ALT. Furthermore; increasing serum albumin and total protein levels and improving hepatic tissue; cell energy status by increasing adenylate energy charge (AEC) and reducing AMP/ATP ratio and preserving hepatic tissue cellular integrity represented by lowering 8-hydroxy-2-deoxyguanosine (8-OHDG); the results were comparable to those of metformin. As a conclusion; using RSV could be valuable in treatment of hepatic complications associated with MetS and its efficacy probably involves enhancement of cell energy as well as conservation of cellular integrity.

INTRODUCTION

Metabolic syndrome (MetS) is considered as an important marker for subsequent development of type 2 diabetes mellitus (T2DM). It is characterized by multiple metabolic abnormalities, including obesity, hypertension, dyslipidemia, insulin resistance (IR), and impaired glucose tolerance. IR and the compensatory hyper-insulinemia are the hallmark of MetS; however, a number of other parameters appear to be related to MetS, including nonalcoholic fatty liver diseases (NAFLDs) (Liaw and Peplow, 2016, Martinez-Abundis *et al.*, 2016). Liver is severely damaged in patients with diabetes mellitus (DM) and/or Mets. These damages include abnormal liver enzymes levels, necrosis, inflammation, cirrhosis, and hepatitis; leading to several nonalcoholic fatty liver diseases (NAFLDs) (Mellendijk *et al.*, 2015). NAFLDs include a spectrum of diseases, ranging from simple fatty liver to non-alcoholic steatohepatitis (NASH), which may progress to hepatocellular carcinoma (Lozano *et al.,* 2016). NAFLD represents the hepatic manifestation of the destruction of the insulin networking and is often characterized by hepatic steatosis; associated with obesity, IR and MetS, which is accompanied by excessive hepatic lipids accumulation. (Bugianesi *et al.,* 2010). There is an increasing evidence that insulin resistant states are accompanied by a low-grade inflammation due to chronic activation of the innate immune system producing a relative excess of pro-inflammatory cytokines such as tumor necrosis factor- α (TNF-α) and interlukin-6 (IL-6), both of which are produced by adipose tissue, thereby leading to an imbalance of pro-inflammatory and anti-inflammatory cytokines (Balistreri *et al.,* 2010, Liaw and Peplow, 2016). Diet supplements including antioxidants, polyunsaturated fatty acids and mineral elements support IR treatment due to their antioxidant and anti-inflammatory properties.

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Moreover, it is considered that a healthy balanced diet enriched with various diet supplements may be the best approach in NAFLD treatment (Sicinska *et al.,* 2015).

Resveratrol (RSV) (3,5,4'-trihydroxystilbene) is a phytoalexin that has been detected in many fruits such as grapes, peanuts and the roots of the Asian plant *Polygonum cuspidatum* (Peredo-Escarcega *et al.,* 2015).RSV was reported to be one of the most powerful nutraceuticals working as an anti-inflammatory and antioxidant as well as hepatoprotective (Chen *et al.,* 2016, Pektas *et al.,* 2016, Radwan and Ahmed, 2016) and recently it has been used as a protective agent against obesity and/or metabolic diseases for example hepatic steatosis and IR; yet results obtained from several studies are still debatable and its exact mechanism of action is still under investigation (Bremer, 2014, Peredo-Escarcega *et al.,* 2015, Vallianou *et al.,* 2013).

High fructose consumption is associated with metabolic disorders including IR and dyslipidemia as well as hepatic steatosis (Morsy *et al.,* 2016). Animal intake of high amounts of fructose and fat is likely to lead to a constellation of abnormalities including IR, hypertriglyceridemia and obesity that mimic human metabolic syndrome. Furthermore; it has been documented that high fat, high fructose diet (HFHF diet) caused enhanced production of free radicals and reduced the antioxidants levels, thereby creating a redox imbalance which eventually results in oxidative stress (Lozano *et al.,* 2016, Maithili Karpaga Selvi *et al.,* 2015, Narasimhan *et al.,* 2015, Rodriguez Lanzi *et al.,* 2016).

The current research aimed to investigate some of the possible mechanisms involved in the efficacy of RSV against IR and hepatotoxic side effects resulting from MetS experimentally induced in rats using HFHF-diet model.

MATERIAL AND METHODS

Animals

Juvenile male albino rats weighing 80-90 g purchased from the animal house at the National Research Centre (NRC, Cairo, Egypt). Upon arrival; the animals were acclimatized for 7 days to a quiet colony room, with controlled ambient temperature (22±1 °C) and a 12 hour natural light/dark cycle, housed eight per cage, fed a standard diet and water was provided *ad lib*. The experiments were performed with 8 rats per treatment group according to a randomized schedule. All experiments were performed according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC)*.*

Drugs

Trans-resveratrol; given as a generous gift from Jing Tea LLC (Australia), as Harmoni-T micronized trans-resveratrol capsules for ingestion. The powder in the capsules was freshly suspended in distilled water just before oral administration. Metformin hydrochloride tablets purchased from Cid Company (Egypt).Tablets were freshly ground and suspended in distilled water just before oral administration.

Experimental design
High fat high fructose insulin resistance model

Rats were weighed and insulin resistance (IR) was induced by adding high-fat diet (60 kcal/100 kcal saturated fat) with 20% fructose in the drinking water for 60 days (Axelsen *et al.,* 2010). A group of 8 rats was used to serve as normal control; kept under the same conditions, fed a standard diet and water was provided *ad lib*. On day 59 rats were fasted over night for 12 hrs except for drinking water (tap water was used for all groups on that night). On Day 60, random rats were chosen from each cage, IR was confirmed by measuring fasting glucose and insulin and by computing fasting insulin sensitivity indices HOMA-IR. At that time; a well-established and permanent IR animal model was present. Starting day 61; all groups except the normal control were daily orally administered their corresponding treatments concomitantly with the high fat high fructose (HFHF) diet. HFHF diet-induced insulin resistant rats were allocated to four groups and were treated as follows: group (1): HFHF control; receiving distilled water (5ml/kg; p.o daily), group (2): Metformin standard group; Metformin standard group; receiving metformin (150 mg/kg; p.o daily), group (3): R20 group; receiving RSV (20 mg/kg; p.o daily), group (4): R40 group; receiving RSV (40 mg/kg; p.o daily). All treatments were given for 10 consecutive days. In addition, the normal control received daily (5ml/kg distilled water p.o). After the last treatment, rats were fasted over night for 12 hrs except for drinking water (tap water was used for all groups on that night). Twenty four hours after the last drugs ingestions; rats were weighed and blood samples were collected. Rats were then sacrificed and liver tissues were isolated and kept at -80 °C for further analyses.

Biochemical assessments
Determination of serum fasting levels of glucose, Insulin and calculation of HOMA-IR

Serum glucose level was determined spectro-photometrically (Trinder, 1969) and the concentration was expressed as mg/dl. Serum insulin level was determined by ELISA kit(Sceti Medical Lab K.K, Tokyo, Japan) the concentration was expressed as µIU/ml (Grassi and Pradelles, 1991). Homeostatic Model Assessment–Insulin Resistance (HOMA-IR) was calculated as follows:

HOMA-IR = [Fasting glucose (mg/dl)/18*Fasting insulin (µIU/ml)]/22.5 (Matthews *et al.,* 1985).

Determination of liver tissue high affinity and low affinity insulin receptors (HAIR & LAIR) expression.

HAIR and LAIR were measured using radio-immunoassay as previously described (Corin and Donner, 1982).

Determination of hepatic triglycerides and total cholesterol

Hepatic triglycerides (TG) and total cholesterol (TC) were measured spectrophotometrically(Fassati,Principe, 1982, Richmond, 1973).

Determination of serum total protein, albumin, ALT and AST levels

Serum total protein, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT) levels were measured spectrophotometrically (Doumas *et al.,* 1971, Gornall *et al.,* 1949, Reitman,Frankel, 1957).

Determination of liver tissue level of GSH, MDA and NOx

Hepatic level of MDA was measured spectro-photometrically (Ruiz-Larrea *et al.,* 1994) and similarly; GSH was measured spectrophotometrically using the method of Ellman (Ellman, 1959) modified by bulaj *et al.*(Bulaj *et al.,* 1998). Hepatic nitric oxide (NOx) products (nitrates and nitrites) levels were determined using ELISA reader.(Miranda *et al.,* 2001)

Determination of liver tissue level of tumor necrosis factor alpha (TNF-α)

The tissue level of tumor necrosis factor alpha (TNF-α) was determined with ELISA kit (Raybiotech) (Bonavida, 1991).

Determination of liver tissue level of 8-OHDG

Isolation and hydrolysis of liver DNA was performed using the method of Lodovici *et al.*(Lodovici *et al.,* 1997). The hydrolyzed mixture was centrifuged and the supernatant were injected into the HPLC. The separation of 8-OHDG was performed with an Agilent HP 1200 series HPLC apparatus (USA). The analytical column was Supelcosil C18 (5 μm particle and 80 A^o pore size) (250 x 4.6 ID). The eluting solution was H_2O/methanol at a ratio (85: 15) with 50 mM KH_2PO_4, pH 5.5 at a flow rate of 0.68 ml/min. the UV detector was set at 245 nm. The resulting chromatogram identified the concentration from the sample as compared to that of the standard purchased from Sigma Aldrich.

Determination of liver tissue ATP, ADP and AMP

The determination of tissue ATP, ADP and AMP was performed with an Agilent HP 1200 series HPLC apparatus (USA). The analytical column was Ultrasphere ODS EC 250 x 4.6 mm column. Mobile phase A consisted of 0.06 mol/l K_2HPO_4 and 0.04 mol/l KH_2PO_4 dissolved in deionized water and adjusted to pH 7.0 with 0.1 mol/l KOH, while mobile phase B consisted of 100 % acetonitrile. Flow rate of the mobile phase was 1.2 ml/min. the UV detector was set at 254 nm. ATP, ADP and AMP in the samples were identified by comparison with standards purchased from Sigma Aldrich (Liu *et al.,* 2006, Teerlink *et al.,* 1993).

Total adenylate energy charge (AEC) was calculated according to the equation:

AEC = (ATP + 0.5ADP)/ (ATP + ADP + AMP)(Atkinson,Walton, 1967).

Statistical analyses

Statistical analyses were carried out using one way ANOVA followed by Tukey's multiple comparisons test. $P<0.05$ was accepted as being significant in all types of statistical tests. Graph prism software (version 6) was used to carry out all statistical tests. Values were expressed as means ± S.E.

RESULTS

Effects of RSV on body weight and metabolic parameters of rats with HFHF diet-induced IR

HFHF showed an elevation the in the body weight as compared to the normal control. Treatment of rats with RSV dose dependently reduced body weight as compared to the HFHF group. Moreover; HFHF was associated with an elevation in the serum fasting glucose and insulin levels as well as HOMA-IR as compared to the normal control. Treatment with RSV dose dependently reduced glucose and insulin levels and HOMA-IR as compared to the HFHF control and the results of the higher dose were comparable to those of metformin. In addition; rats fed with HFHF diet showed significant elevation in liver tissue TG and TC levels as compared to the normal control. Treatment with RSV dose dependently reduced TG level significantly as compared to the HFHF control and furthermore; normalized TC level (Table 1).

Table 1: Effects of RSV on body weight and metabolic parameters of rats with HFHF diet-induced IR.

Groups	Body weight (g)	Serum glucose mg/dl	Serum insulin μIU/ml	HOMA-IR	Hepatic TG mg/g tissue	Hepatic TC mg/g tissue
Normal	107.40±3.58[*]	62.63±1.13[*]	1.58±0.06[*]	0.23±0.01[*]	142.3±6.03[*]	15.87±0.75[*]
HFHF	192.80±5.78[#]	94.82±1.56[#]	16.78±0.45[#]	3.94±0.16[#]	309.9±22.19[#]	34.32±1.09[#]
Metformin	142.00±3.21[*#]	67.13±2.04[*]	5.51±0.17[*#]	0.92±0.05[*#]	150.8±6.92[*]	21.57±1.07[*#]
R20	169.40±2.99[*#]	82.3±1.34[*#]	9.46±0.26[*#]	1.93±0.07[*#]	206.2±11.47[*#]	17.4±0.68[*]
R40	155.00±3.06[*#]	68.38±2.17[*]	5.61±0.18[*#]	0.95±0.05[*#]	205.9±12.22[*#]	16.06±0.20[*]

*Significantly different from HFHF control, # significantly different from normal control at p<0.05.
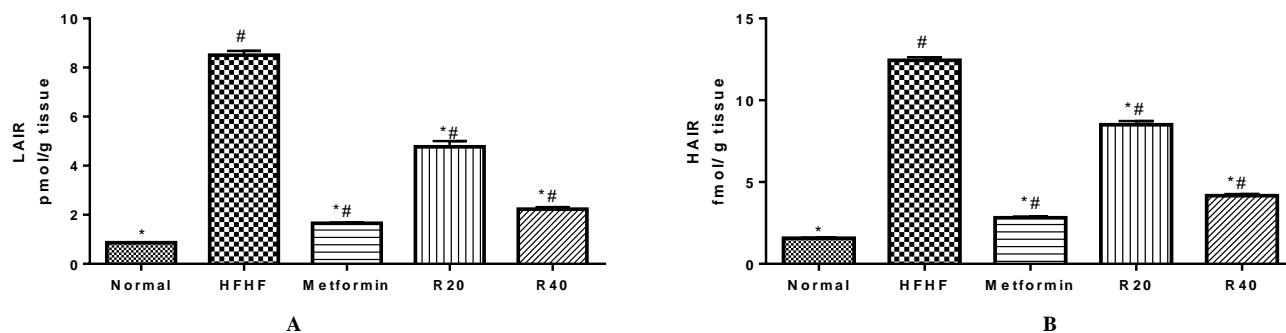
Fig. 1: Effects of RSV on hepatic LAIR (a) and HAIR (b) expression in rats with HFHF diet-induced IR.
*Significantly different from HFHF control, # significantly different from normal control at p < 0.05.

Table 2: Effect of RSV on serum ALT, AST, albumin and total protein in rats with HFHF diet-induced IR.

Groups	ALT u/l	AST u/l	Albumin mg/dl	Total protein mg/dl
Normal	24.66 ± 0.99*	133.34 ± 3.29*	4.57 ± 0.16*	7.79 ± 0.30 *
HFHF	88.92 ± 4.56#	202.67 ± 7.48#	3.61 ± 0.04#	5.49 ± 0.34 #
Metformin	52.20 ± 1.61*#	170.77 ± 10.16*#	3.82 ± 0.06#	7.40 ± 0.23 *
R20	75.61 ± 2.79*#	191.33 ± 3.88#	4.23 ± 0.16 *	7.48 ± 0.63*
R40	48.91 ± 4.03*#	160.68 ± 2.42*#	4.28 ± 0.16 *	7.66 ± 0.65 *

*Significantly different from HFHF control, # significantly different from normal control at p < 0.05.

Table 3: Effects of RSV on hepatic oxidative and nitrosative stresses biomarkers in rats with HFHF diet-induced IR.

Groups	GSH ug/g	MDA nmol/g	NOx umol/g
Normal	29.12 ± 1.116*	113.20 ± 3.25*	19.39± 0,78*
HFHF	23.00 ± 0.48#	208.7 ± 1.24#	88.58±4.74#
Metformin	26.45 ± 0.52*#	122.8 ± 5.70*	59.56±3.18*#
R20	26.05 ± 0.29*#	161.1 ± 11.8*#	70.57± 1.54*#
R40	26.3 ± 0.2 5*#	154.1 ± 4.49*#	53.30±2.22*#

*Significantly different from HFHF control, # significantly different from normal control at p <0.05.

Effects of RSV on hepatic LAIR and HAIR expression in rats with HFHF diet-induced IR

Induction of IR by feeding rats with HFHF diet showed a marked increase in the expression of hepatic LAIR and HAIR in liver tissue (8.51±0.17 vs. 0.86±0.02 pmol/g and 12.45±0.18 vs. 1.57±0.03 fmol/g) respectively as compared to the normal control. Oral treatment of rats with RSV(20 and 40 mg/kg; p.o.) reduced the expression of hepatic LAIR (4.77±0.23 and 2.23±0.08 *vs.* 8.51±0.17 pmol/g) and HAIR (8.51±0.22 and 4.17±0.09 vs. 12.45±0.18 fmol/g) in a dose dependent manner as compared to HFHF control group.

This modulatory effect was comparable to those of metformin (Figure 1).

Effect of RSV on serum ALT, AST, albumin and total protein in rats with HFHF diet-induced IR

High fat high fructose diet-induced IR significantly increased serum ALT and AST as compared to the normal control. Treatment of rats with RSV showed a prominent decrease in serum ALT and AST contents as compared to the HFHF control and the results were comparable to those of metformin. Similarly, HFHF diet-induced IR significantly decreased serum albumin and total protein as compared to the normal control. Treatment of rats with

RSV elevated serum albumin and total protein in dose dependent manner as compared to the HFHF control and moreover: the results were more prominent over those of metformin (Table 2).

Effects of RSV on hepatic oxidative and nitrosative stresses biomarkers in rats with HFHF diet-induced IR

HFHF significantly decreased liver GSH and significantly increased liver MDA and NOx levels as compared to the normal control. Treatment of rats with RSV reduced liver MDA and NOx levels as compared to the HFHF control. Moreover; RSV significantly elevated liver GSH content and the results were comparable to those of metformin (Table 3).

Effects of RSV on liver level of TNF-α in rats with HFHF diet induced IR

Induction of IR was accompanied with a marked increase in hepatic TNF-αcontent (641.1 ± 10.82*vs*. 425.9 ± 10.36 pg/g tissue) as compared to the normal control*P*< 0.05. Treatment with both metformin and RSV (20 mg/kg) resulted in significant reduction in the TNF-α level as compared to the HFHF group (585.8 ± 13.69 and 579.1 ± 7.836 vs. 641.1 ± 10.82 pg/g tissue) at *P*< 0.05. On the other hand, RSV (40 mg/kg) normalized TNF-α level (Figure 2).
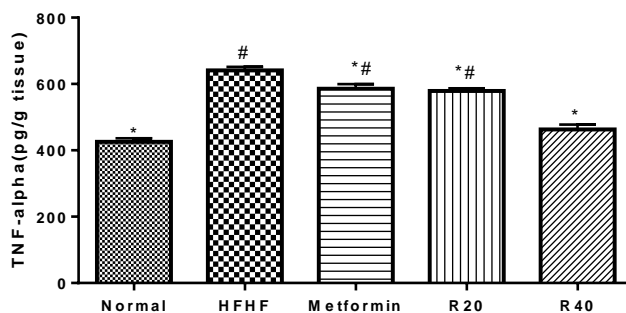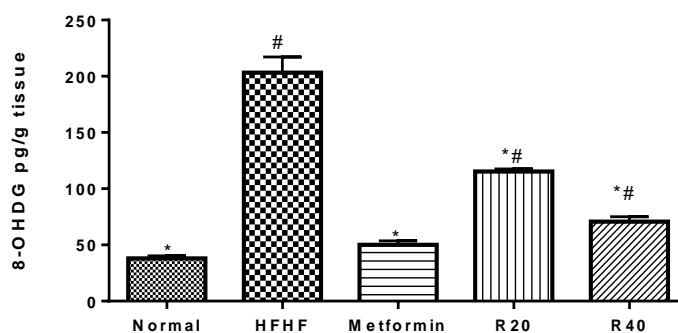
Fig. 2: Effects of RSV on liver level of TNF-α in rats with HFHF diet-induced IR.
*Significantly different from HFHF control, # significantly different from normal control at p < 0.05.

Table 4: Effects of RSV on liver tissue cell energy in rats with HFHF diet-induced IR.

Groups	ATP ug/g tissue	ADP ug/g tissue	AMP ug/g tissue	AEC	AMP/ATP
Normal	56.11 ± 0.92 [*]	31.82 ± 1.18 [*]	24.05 ± 1.02 [*]	0.643 ± 0.007 [*]	0.43 ± 0.02 [*]
HFHF	25.66± 0.28 [#]	14.95 ± 0.27 [#]	14.49 ± 0.77 [#]	0.602 ±0.009 [#]	0.57 ± 0.03 [#]
Metformin	44.26 ± 1.31 [*#]	25.47 ± 0.77 [*#]	19.44 ± 0.53 [*#]	0.618 ± 0.006	0.44 ± 0.02 [*]
R20	31.40 ± 1.04 [*#]	20.66 ± 0.67 [*#]	15.44 ± 0.34 [#]	0.637 ± 0.001 [*]	0.50 ± 0.02
R40	38.97 ± 0.84 [*#]	25.31 ± 0.42 [*#]	16.80 ± 0.41 [#]	0.639 ± 0.007 [*]	0.43 ± 0.01 [*]

*Significantly different from HFHF control, [#] significantly different from normal control at p < 0.05.

Fig. 3: Effects of RSV on liver tissue 8-OHDG content in rats with HFHF diet-induced IR.
*Significantly different from HFHF control, # significantly different from normal control at p < 0.05.

Effects of RSV on liver tissue cell energy in rats with HFHF diet-induced IR

HFHF resulted in significant reduction in the cell energy represented by the lowered ATP, ADP and AMP, AEC levels and increased AMP/ATP ratio as compared to the normal control. RSV at its two dose levels (20 and 40 mg/kg) and metformin significantly elevated the cell energy parameters as compared to the HFHF control at p<0.05 where the overall results of resveratrol (40 mg/kg) were prominent over metformin (Table 4).

Effects of RSV on liver tissue 8-OHDG content in rats with HFHF diet-induced IR

HFHF resulted in significant elevation in 8-OHDG content in the liver tissue suggesting severe tissue damage and DNA fragmentation (203.3 ± 13.85 *vs.* 38.1 ± 2.22 pg/g) as compared to normal control. RSV(20 and 40 mg/kg) treatment significantly reduced the 8-OHDG level (115.3 ± 2.23 and 70.81 ± 4.26 *vs.* 203.3 ± 13.85 pg/g) respectively as compared to the HFHF

control and the results were comparable to those of metformin. (Figure 3).

DISCUSSION

Insulin resistance (IR), the hallmark of MetS, is a metabolic condition in which the cells do not use insulin properly and as the need for insulin rises and the pancreas gradually loses its ability to produce it; the condition usually leads to type 2 diabetes mellitus (T2DM) (Kozono *et al.,* 2016). It is usually characterized by the impairment of glucose uptake in muscle and the elevation of endogenous glucose production by the liver resulting in hyperglycemia. IR is accompanied with severe liver damage with abnormal liver enzymes levels and hepatic inflammation (Mellendijk *et al.,* 2015).

High fat-high fructose diet (HFHF) is now considered as a well-established model for the induction of IR in rodents (Wang *et al.,* 2015a, Zhang *et al.,* 2016, Zhuhua *et al.,* 2015). In

accordance with previous studies (Afifi *et al.,* 2016, Lozano *et al.,* 2016), the present study confirms the relationship between HFHF diet-induced IR model in rats and its impact on hepatic tissue such as hepatic steatosis complicated by fibrosis, inflammation, and oxidative stress.

In our study; adding high fat in the diet and high fructose in the drinking water of rats was associated with a marked increase in body weight and HOMA-IR indicating the development of insulin resistance in rats. Moreover; the model lead to amplification in HAIR and LAIR expression in liver tissue, significant increase in serum liver functions *viz.* ALT and AST, as well as liver tissue TC and TG levels, decrease in serum total protein and albumin levels along with liver tissue marked oxidative and nitrosative stresses, elevation in liver tissue TNF-α level, liver tissue DNA fragmentation as demonstrated by the elevated 8-OHDG level and severe reduction in liver tissue cell energy represented by lowered adenylate energy charge (AEC) level and increased AMP/ATP ratio; all of which are markers of hepatic injury.

Hepatocytes have a complicated system of enzymatic and non-enzymatic antioxidant defenses to neutralize reactive oxygen and nitrogen species (ROS) and (RNS). However, increased levels of ROS and RNS may overcome the hepatocellular antioxidant defense mechanism, resulting in hepatocyte injury and inducing hepatic steatosis which subsequently end with cell death (Lozano *et al.,* 2016).Liver in an insulin-dependent tissue and plays a vital role in glucose and lipid homeostasis (Sivajothi *et al.,* 2007). High fasting blood glucose level is considered as a stamp characteristic of IR resulting from loss of insulin sensitivity in its target tissues(Reddy *et al.,* 2016). Fluctuating levels of AST and ALT are mostly the result of the leakage of these enzymes from the cytosol of hepatocytes into the blood stream. However, increased levels of these enzymes may result in low levels of serum albumin and total protein that are used as indicators of liver functions.A decrease in serum albumin and total protein levels may contribute to the inhibition of oxidative phosphorylation process, leading to a reduction in protein absorption, a decline in protein synthesis, and an increase in the catabolic process (Ghanbari *et al.,* 2016).

Furthermore, our results are in accordance with the two-hits hypothesis described by Day and James (Day,James, 1998), in which the accumulation of hepatic TG constitutes the first hit of NASH pathogenesis, and oxidative stress followed by inflammation which represents the second hit. The development of hepatic steatosis via accumulation of TG in hepatocytes increases the vulnerability of the liver to inflammation, fibrosis, and cellular death representing hallmarks of NASH.

In addition; it has been shown that obesity in rodents resulted in an increase in macrophages in adipose tissue due to an increased infiltration and/or proliferation of cells (Mellendijk *et al.,* 2015, Patel *et al.,* 2016). Besides; IR is accompanied with increased numbers of classically activated macrophages in the white adipose tissue producing many pro-inflammatory cytokines like TNF-α, IL-1, and IL-6(Mellendijk *et al.,* 2015). IR promotes DNA damage via the induction of oxidative stress (Paneni *et al.,*

2013).Therefore, antioxidants protect cells against oxidative damage. Decrease in antioxidant defense system is followed by the damage of cellular organelles and enzymes, increased level of lipid peroxidation, and development of IR. Oxidative and nitrosative stresses causing structural and functional alterations in the cellular biomolecules and cell membrane are the result of the development of complications in diabetic individuals(D'Archivio *et al.,* 2012, Fisher-Wellman,Bloomer, 2009, Park *et al.,* 2009). Attack by ROS and RNS leads to DNA hydroxylation; oxidizing DNA to form 8-hydroxy-2-deoxyguanosine (8-OHDG) adducts; a major species of oxidative DNA damage (Aksit,Bildik, 2014). 8-OHDG content is considered a sensitive biomarker of the oxidative DNA damage and repair (Abdelali *et al.,* 2016).

Tissue HAIR and LAIR receptors expression could be used as reliable method of determining the ability of body organs to utilize insulin. Increase in the number of insulin receptors, usually indicates impaired insulin utilization and relates positively with hyper-insulinemia and IR and negatively with insulin sensitivity (Amin *et al.,* 2014).

Finally; tissue ATP, ADP and AMP levels reveal the energetic status of cells. Nowadays; adenylate energy charge (AEC) equation is used to determine the cell energy status (De la Fuente *et al.,* 2014). Diminution of energy insufficiencies designated by elevated ATP and adenylate energy charge levels, and decreased AMP/ATP ratio could be used as consistent markers demonstrating protection against tissue injury (Wang *et al.,* 2015b).

Recently; resveratrol (RSV) has been investigated for the possibility of having anti-hyperglycemic and hepatoprotective actions in several animal models for diabetes as well as clinical studies on diabetic and/or healthy volunteers alone or concomitant with high fat diet regimen, with or without anti-diabetic medications. Yet the results have been so far controversial and the mechanism of action has not been totally clarified (Timmers *et al.,* 2012, Zeng *et al.,* 2016, Zhao *et al.,* 2016). The main findings were mainly that in some studies RSV improved glycemic control by mechanisms that involve improvement in insulin secretion and activity and sometimes anti-inflammatory and hepatoprotective effects (Pektas *et al.,* 2016, Yonamine *et al.,* 2016, Zare Javid *et al.,* 2016). In the present study, we focused on the effect of RSV on hepatic tissue cells energy state along with the ability to maintain cells integrity by prevention of cellular DNA fragmentation and improvement of the liver tissue ability to utilize insulin. Results of the study revealed improvement in IR and the concomitant liver ability to utilize insulin which was represented by reduced HOMA-IR as well as decreased liver HAIR and LAIR expression. Enhancement of liver performance in addition to amelioration in liver tissue damage were also detected through serum and tissue liver functions, inflammatory, DNA fragmentation and cell energy biomarkers signified by reduced ALT, AST, TC and TG levels and increased albumin and total protein levels, decreased TNF-α, 8-OHDG contents, elevated adenylate energy charge (AEC) along with lowered AMP/ATP ratio.Hepatic oxidative and nitrosative stresses also declined. The

overall results were comparable to those of metformin indicating the beneficial effects of using RSV in IR.

CONCLUSION

Using RSV as daily supplement could be advantageous in ameliorating hepatic insult associated with IR in MetS. The mechanism of action of RSV possibly involves restoration of cell energy, preservation of cell integrity and prevention of DNA fragmentation.

FUNDING SOURCE

CONFLICTS OF INTEREST

The authors declare there were no conflicts of interest

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