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Correlation between brain neurotransmitters and insulin sensitivity: Neuro-preservative role of resveratrol against high fat, high fructose-induced insulin resistance

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ABSTRACT

Insulin resistance (IR) is a major public health problem that can lead to many dangerous medical disorders and early mortality. This study aimed to explore the effectiveness of resveratrol (RSV) to counteract the neuro-complications accompanying high fat, high fructose (HFHF) diet experimentally induced-IR in rats. IR was induced by the ingestion of HFHF diet for 70 days, 80 juvenile rats were used, and the treatments were given orally for the diet latest 10 days. Rats' general behavior was assessed by open field test (OFT) and forced swimming test (FST). On biochemical level; neuro-complications were assessed by measuring brain levels of monoamines and their metabolites as well as the levels of 8-hydroxyguanosine (8-OHDG), tumor necrosis factor-α (TNF-α), malondialdehyde (MDA), reduced and oxidized glutathione (GSH and GSSG) and nitric oxide (NO_x). Oral RSV (20 and 40 mg/kg *p.o*) increased the activity of the rats in the OFT and decreased the immobility period in the FST in a dose-dependent manner. Moreover, RSV reduced monoamines turnover, elevated GSH, and reduced GSSG, NO_x, MDA, 8-OHDG, and TNF-α ($p < 0.05$). RSV exhibited neuro-protective activities against HFHF-induced IR, thus it can be recommended as a favorable daily dietary supplement for treating the neuronal side effects related to IR.

INTRODUCTION

The incidence of depressive or anxiety disorders is up to 60%–80% higher in patients with type 2 diabetes mellitus (T2DM) than in the healthy population. This could be partially explained by the fact that one of the key precursors of T2DM is the presence of an insulin-resistant state, and previously it was demonstrated that one of the major neurodegenerative disorders that comorbid with insulin resistance (IR) was depression (Kahl *et al*., 2015; Li *et al*., 2013; Ryan *et al*., 2012).

In the last decades, the prevalence of pediatric overweight and obesity has increased reaching 20% or more in some countries and is frequently associated with impaired IR (Lalanza *et al*., 2014). Insulin is a hormone that plays a major role in maintaining the homeostasis between glucose uptake and production. IR is a metabolic condition known to be a state of reduced response to insulin in target tissues, the urge for insulin increases, and the pancreas gradually fails to produce it (Afifi *et al*., 2017; Kim *et al*., 2015). The consequences include deficits in energy metabolism, increased inflammation and oxidative stress, and could lead to cellular degeneration and death (De La Monte, 2012). In the central nervous system (CNS), insulin regulates a broad array of functions as cell growth and survival, synapse formation, neurotransmitter function, and plasticity. Chronic IR has adverse consequences on the functional integrity of the CNS, including reduced receptor responsiveness and inhibition of downstream signaling (Arnold *et al*., 2014).

Oxidative damage plays a vital part in the pathogenesis of IR-induced neuronal degeneration, which highlights the significance of antioxidants in the treatment of IR associated neurodegenerative disorders (Li *et al*., 2018; Yin *et al*., 2013). Recently, several dietary supplements have been suggested

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in the management of IR due to their antioxidant and anti-inflammatory activities (Bamagous *et al*., 2018; Sicinska *et al*., 2015).

Resveratrol (RSV; 3,5,4′-trihydroxystilbene) is a polyphenolic compound with a broad spectrum of pharmacological properties. It has been reported to act as an anti-inflammatory, antioxidant hepatoprotective and neuroprotective (Ahmed-Farid *et al*., 2016). Evidence abound indicating the usefulness of RSV in the treatment of several metabolic complications including metabolic syndrome, obesity, T2DM, and hyperglycemia (Ahmed-Farid *et al*., 2016; Radwan *et al*., 2016).

The present study investigated the beneficial effects of RSV as a dietary supplement to antagonize the neuro-complications associated with high fat, high fructose diet (HFHF)-induced IR in rats. In addition, metformin (MT) served as a standard anti-diabetic drug with neuroprotective potentials.

MATERIAL AND METHODS

Animals used

Juvenile 5-weeks-aged male albino rats (Lalanza *et al*., 2014) weighing 80–90 g were obtained from the animal house at the National Research Centre (NRC, Cairo, Egypt). Animals were then allowed to acclimatize in a quiet room (eight rats/cage), with controlled ambient temperature (22°C ± 1°C) and a 12 hours light/dark cycle, fed a standard diet, and water was provided *ad lib*. The animals were allocated randomly, whereas each group containing 16 rats/treatment group.

Ethics statement

This study was achieved according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH No. 85:23 revised 1985) in unity with the guidelines provided by the World Medical Association Declaration of Helsinki on Ethical Principles for studies concerning experimental animals.

Drugs

Trans-resveratrol (Jing Tea LLC, Australia), as Harmoni-T micronized trans-resveratrol capsules for ingestion were freshly suspended in distilled water prior to oral administration. Metformin hydrochloride tablets (Cid Company, Egypt) were freshly ground and suspended in distilled water just before oral administration.

Experimental design

High fat, high fructose (HFHF) insulin resistance model

Eighty rats were weighed and allocated in equal groups (16 rats each). One group served as normal control; fed standard diet and tap drinking water. IR was induced in the remaining 64 rats using a high-fat diet; 60 kcal/100 kcal saturated fat with 20% fructose in the drinking water for 60 days (Axelsen *et al*., 2010). On day 60; rats were fasted overnight and IR was confirmed by computing homeostatic model assessment of insulin resistance (HOMA-IR). HFHF diet-induced insulin-resistant rats were allocated to four groups and were treated as follows: group (1): HFHF control; receiving distilled water (5 ml/kg; p.o daily), group (2): MT standard group; receiving metformin (MT; 150 mg/kg/day; p.o) for 10 consecutive days, group (3): R20 group; receiving RSV (20 mg/kg/day; p.o) for 10 consecutive days, and group (4): R40 group; receiving RSV (40 mg/kg/day; p.o) for 10 consecutive days. In addition to a normal control receiving 5 ml/kg distilled water/day; p.o). On day 70, eight rats from each group were randomly selected for the conduction of behavioral tests, 1 hour after the last treatments. Twenty-four hours after the last drug ingestions, overnight fasted rats from each group were randomly divided into two subgroups (eight rats each). The first subgroup was used for the collection of blood samples under phenobarbital anesthesia to assess serum; glucose, insulin, and compute the final effect of the treatments on HOMA-IR. The second subgroup was sacrificed by decapitation, for the collection of brain tissues which were then isolated and kept at 80°C for further investigation.

Behavioral assessments

Open field test (OFT)

Open field test was performed in a square wooden arena (80 × 80 × 40 cm high). The test was conducted according to Ahmed *et al*. (2014a). Ambulation frequency: number of squares crossed by the animal and rearing frequency: number of times the animal stood stretched on its hind limbs with or without forelimb support were calculated (Ahmed *et al*., 2014a).

Forced swimming test (FST)

According to Porsolt *et al*. (1977); forced swimming test was implemented in a cylindrical water tank (70 cm high, 40 cm diameter) (Porsolt *et al*., 1977).

Biochemical assessments

Determination of serum fasting levels of glucose and insulin

Serum levels of glucose and insulin were determined spectrophotometrically (Trinder, 1969) and by ELISA kit (Sceti Medical Lab K.K, Tokyo, Japan) (Grassi *et al*., 1991), respectively. 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).

High performance liquid chromatography (HPLC) measurements

All produced chromatogram identified the concentration from the sample in comparison with that of the corresponding standard purchased from Sigma Aldrich.

Determination of brain tissue level of monoamines and their metabolites (μg/g tissue) levels

Brain monoamines and their metabolites were detected by high performance liquid chromatography (HPLC) [mobile phase; 20 mM potassium phosphate, pH 2.5: methanol (99:1), flow rate; 1.5 ml/minute, *Ultraviolet* (UV); 210 nm]. (Pagel *et al*., 2000).

Determination of brain tissue reduced glutathione (GSH) (µmol/g tissue) and oxidized glutathione (GSSG) (µmol/g tissue) levels

The thiols compounds of oxidized and reduced glutathione (GSH and GSSG) were detected by HPLC (mobile phase; 0.0025 M sodium phosphate buffer, pH 3.5, containing 0.005 M tetrabutylammonium phosphate and 13% methanol, flow rate; 1 ml/minute, UV; 190 nm) (Jayatilleke *et al*., 1993; Yoshida, 1996).

Determination of brain tissue malondialdehyde (MDA) (nmol/g tissue) level

For determination of malondialdehyde (MDA) level; the samples were analyzed using HPLC [mobile phase; 82.5:17.5 (v/v) 30 mM monobasic potassium phosphate (pH 3.6)–methanol, flow rate; 1.2 ml/minute, UV; 250 nm] (Karatas *et al*., 2002; Karatepe, 2004; Lazzarino *et al*., 1991).

Determination of brain tissue nitric oxide (NOx) (µmol/ g tissue) level

Nitric oxide (NO_x) level was determined using HPLC [mobile phase; mixture of 0.1 M NaCl–methanol (45:55), flow rate; 2 ml/minute, UV; 230 nm] (Papadoyannis *et al*., 1999).

Determination of brain tissue 8-hydroxyguanosine (8-OHDG) (pg/g tissue) level

The separation of 8-OHDG was performed with HPLC [eluting solution; H_2O/methanol at a ratio (85:15) with 50 mM KH_2PO_4, pH 5.5; flow rate; 0.68 ml/minute, UV; 245 nm] (Lodovici *et al*., 1997).

Determination of brain tissue level of tumor necrosis factor alpha (TNF-α) (pg/g tissue)

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

Statistical Analysis

Statistical analysis was performed by non-parametric K independent samples Kruskal–Wallis test then Dunn's multiple comparisons test for the open field test (OFT) was performed. Comparisons between means for all other parameters were performed using one-way analysis of variance followed by Tukey's multiple comparisons test at $p < 0.05$. GraphPad Prism software (version 6) was used and values were expressed as Mean ± SE. Where *significantly different from the normal control group,@ significantly different from HFHF control group at $p < 0.05$.

Table 1. Effects of resveratrol on open field test parameters.

Groups	Ambulation (Count/ 5 minutes)	Rearing (Count/5 minutes)
Normal	43.75 ± 2.52	24.5 ± 1.04
HFHF	25.5 ± 1.88*	10.75 ± 0.88*
Metformin	45.5 ± 2.26@	23.63 ± 1.57@
R20	39.13 ± 2.59	19.5 ± 0.63
R40	47.75 ± 2.39@	25.63 ± 1.00@

*Significantly different from the normal control group, @significantly different from HFHF control group at $p < 0.05$.

RESULTS

Behavioral assessments

Effects on open field test parameters

Adding HFHF to the diet of rats resulted in a significant reduction in both ambulation and rearing frequencies in comparison with normal control. RSV (20 mg/kg) resulted in a slight increase in both frequencies yet results obtained were statistically non-significant from the HFHF group. On the other hand, RSV (40 mg/kg) normalized both frequencies and the results were nearly equivalent to those of MT (Table 1).

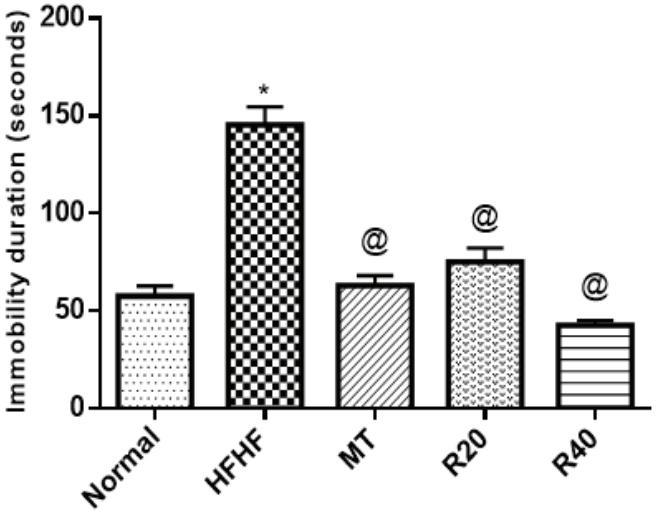


Figure 1. Effect of resveratrol on the immobility period in the forced swimming test. *Significantly different from normal control group, @ significantly different from the HFHF control group at $p < 0.05$.

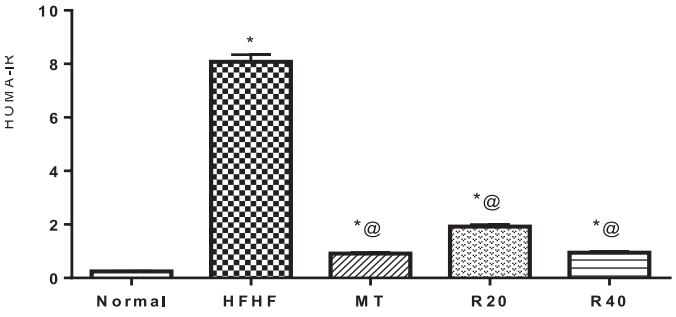


Figure 2. Effect of resveratrol on serum HOMA-IR. *Significantly different from the normal control group, @ significantly different from the HFHF control group at $p < 0.05$.

Effects on immobility period in the forced swimming test

Adding HFHF to the diet of rats resulted in a significant elevation in the immobility period in the forced swimming test (FST) (145.4 ± 9.18 seconds vs. 57.5 ± 5.18 seconds) in comparison with normal control. RSV dose-dependently resulted in a significant decrease in the immobility period (75.00 ± 7.32 seconds and 42.5 ± 2.50 seconds vs. 145.4 ± 9.18 seconds), respectively, when compared with HFHF control group and the outcomes of the high dose approached those of MT (Fig. 1).

Effects on serum HOMA-IR

High fat, high fructose diet group significantly elevated HOMA-IR when compared to the normal control group. Treatment with RSV dose-dependently reduced HOMA-IR in comparison with the HFHF control. The higher dose of RSV outcomes was similar to MT (Fig. 2).

Effects on brain monoamines and their metabolites

Adding HFHF to diet to rats significantly increased serotonin [5-hydroxytryptamine (5-HT)] and dopamine (DA) turn over in comparison with normal control where both 5-HT and DA levels were decreased and the levels of their metabolites; 5-hydroxyindoleacetic acid, 3,4-dihydroxyphenylacetic acid, and homovanillic acid were increased. Furthermore, the DA/norepinephrine (NE) ratio was shifted toward the NE side. R20 and R40 significantly reduced 5-HT and DA turn over and shifted the DA/NE ratio toward the formation of DA in comparison with HFHF control while the effect of the higher dose reached that of MT (Fig. 3).

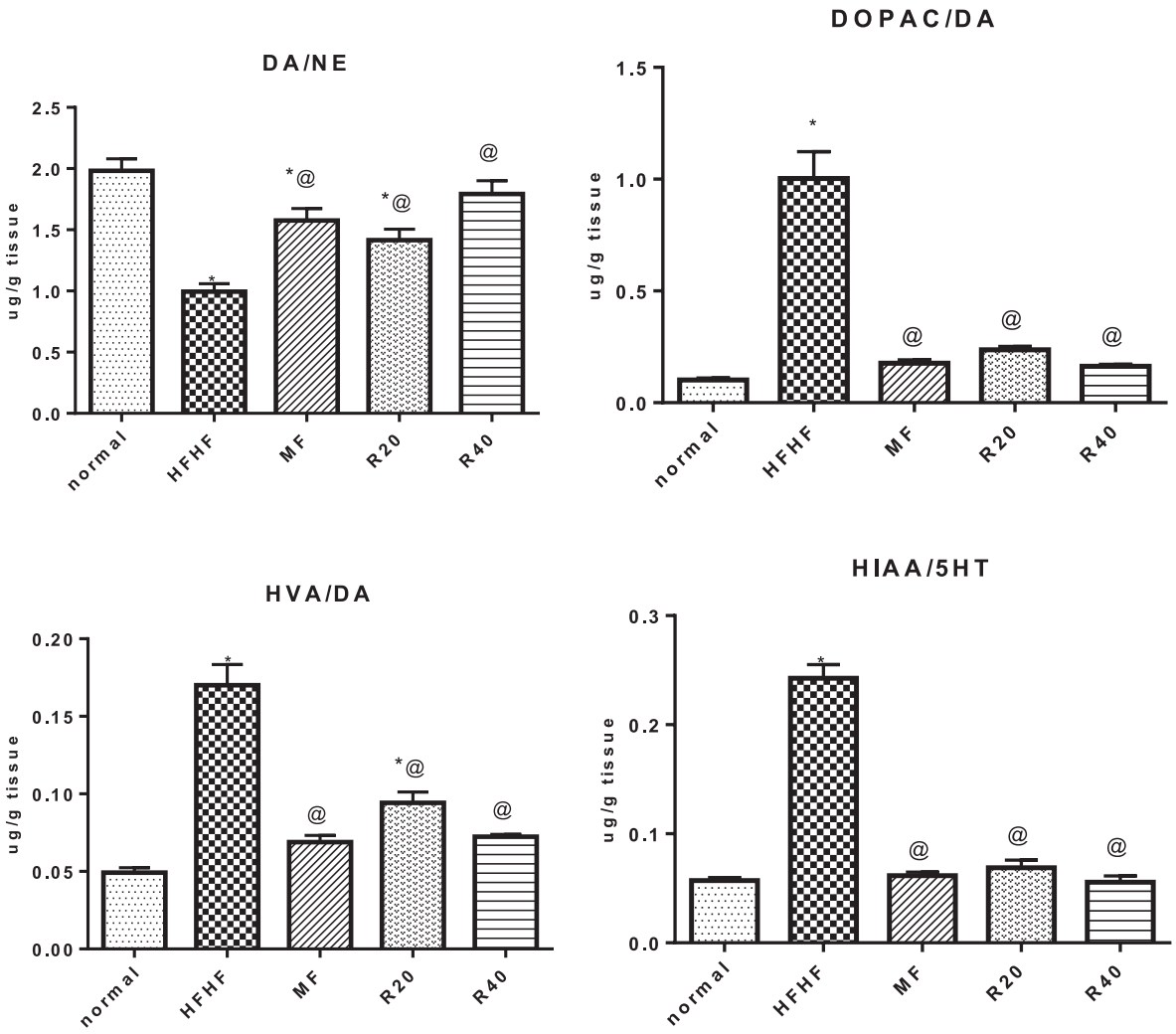


Figure 3. Effect of resveratrol on brain monoamines turnover. *Significantly different from the normal control group, @ significantly different from the HFHF control group at $p < 0.05$.

Table 2. Effects of resveratrol on brain oxidative and nitrosative stresses.

Groups	MDA (nmol/g)	NOx (umol/g)	GSSG/GSH (umol/g)
Normal	68.23 ± 1.37	29.53 ± 0.64 [@]	0.103 ± 0.003
HFHF	111.9 ± 3.39[*]	42.89 ± 1.15[*]	0.258 ± 0.01[*]
Metformin	82.1 ± 1.35[*@]	34.19 ± 0.55[*@]	0.162 ± 0.005[*@]
R20	88.38 ± 1.76[*@]	38.96 ± 0.59[*@]	0.172 ± 0.003[*@]
R40	78.88 ± 1.92[*@]	32.32 ± 0.76[@]	0.151 ± 0.004[*@]

[*]Significantly different from the normal control group, [@]significantly different from HFHF control group at $p < 0.05$.

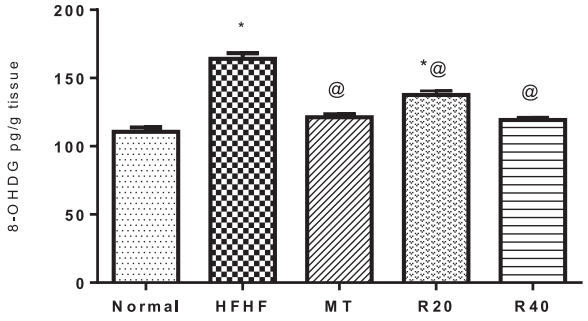


Figure 4. Effect of resveratrol on brain tissue level of 8-OHDG. [*]Significantly different from the normal control group, [@]significantly different from the HFHF control group at $p < 0.05$.

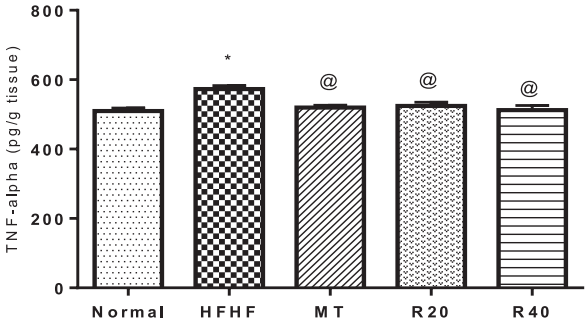


Figure 5. Effect of resveratrol on brain tissue level of tumor necrosis factor alpha (TNF-α). [*]Significantly different from the normal control group, [@]significantly different from HFHF control group at $p < 0.05$.

Effects on brain oxidative and nitrosative stresses

Insulin resistance resulted in a significant elevation in MDA and NOx levels as well as GSSG/GSH ratio indicating pronounced oxidative and nitrosative stresses in comparison with normal control. RSV dose-dependently reduced both the oxidative and nitrosative stresses (Table 2).

Effects on brain tissue level of 8-OHDG

Insulin resistance significantly increased the brain tissue level of 8-OHDG indicating pronounced DNA fragmentation (164.1 ± 4.14 vs. 110.6 ± 3.13 pg/g tissue) when compared with the normal control group. RSV at the low dose decreased 8-OHDG level (137.6 ± 3.00 vs. 164.1 ± 4.14 pg/g tissue) in comparison with the HFHF group. Moreover, RSV at the high dose-normalized 8-OHDG level which was comparable to those of MT (Fig. 4).

Effects on brain tissue level of tumor necrosis factor alpha (TNF-α)

HFHF-induced IR prominently elevated in the brain tissue TNF-α level (573.1 ± 7.91 vs. 509.8 ± 6.33 pg/g tissue) in comparison with normal control. RSV at the two dose levels normalized TNF-α level (524.6 ± 10.22 and 512.6 ± 13.25 pg/g tissue), respectively (Fig. 5).

Pearson's correlations studies

Correlation studies demonstrated that there was a direct significant positive correlation between HOMA-IR and the increased TNF-α level, 8-OHDG in addition to oxidative and nitrosative stresses. Moreover, there was a negative correlation between HOMA-IR and DA as well as serotonin levels. TNF-α proved to be negatively correlated to the serotonin level. 8-OHDG exhibited a negative correlation with both DA and serotonin levels and positive correlation to oxidative and nitrosative stresses. Finally, oxidative and nitrosative stresses were negatively correlated to both DA and serotonin levels (Table 3, Fig. 6).

Table 3. Pearson's correlations studies.

	TNF-α	8-OHDG	DA	Serotonin	MDA	NO	GSH	GSSG	HOMA
HOMA	0.668[**]	0.880[***]	−0.533[*]	−0.924[***]	0.886[***]	0.784[**]	−0.660[**]	0.838[***]	NA
TNF-α	NA	0.614[**]	−0.326	−0.647[**]	0.667[**]	0.473[*]	−0.364	0.587[*]	0.668[**]
8-OHDG	0.614[**]	NA	−0.607[**]	−0.814[***]	0.810[***]	0.891[***]	−0.647[**]	0.809[***]	0.880[***]
MDA	0.667[**]	0.810[***]	−0.653[**]	−0.804[***]	NA	0.802[***]	−0.683[**]	0.892[***]	0.886[***]
NO	0.473[*]	0.891[***]	−0.648[**]	−0.716[***]	0.802[***]	NA	−0.616[**]	0.796[**]	0.784[**]
GSH	−0.364	−0.647[**]	0.886[***]	0.585[**]	−0.683[**]	−0.616[**]	NA	−0.646[**]	-0.660[**]
GSSG	0.587[*]	0.809[***]	−0.617[**]	−0.836[***]	0.892[***]	0.796[**]	−0.646[**]	NA	0.838[***]
DA	−0.326	−0.607[**]	NA	0.428[*]	−0.653[**]	−0.648[**]	0.886[***]	−0.617[**]	-0.533[*]
Serotonin	−0.647[**]	−0.814[***]	0.428[*]	NA	−0.804[***]	−0.716[**]	0.585[**]	−0.836[***]	-0.924[***]

Significant correlation at ($p < 0.05$)*, ($p < 0.01$)**, ($p < 0.001$)***. − = negative correlation, NA = non-applicable correlation.

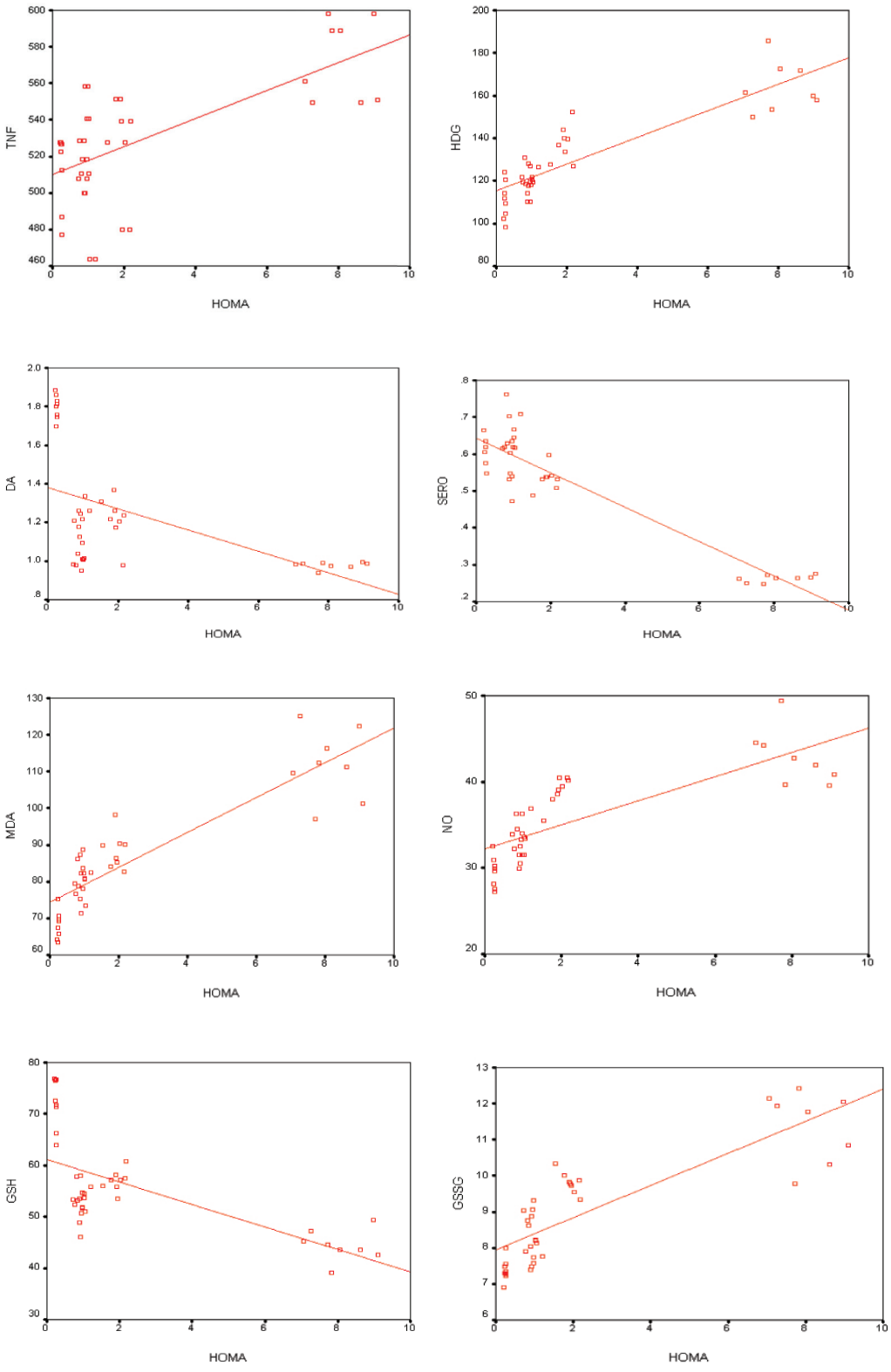
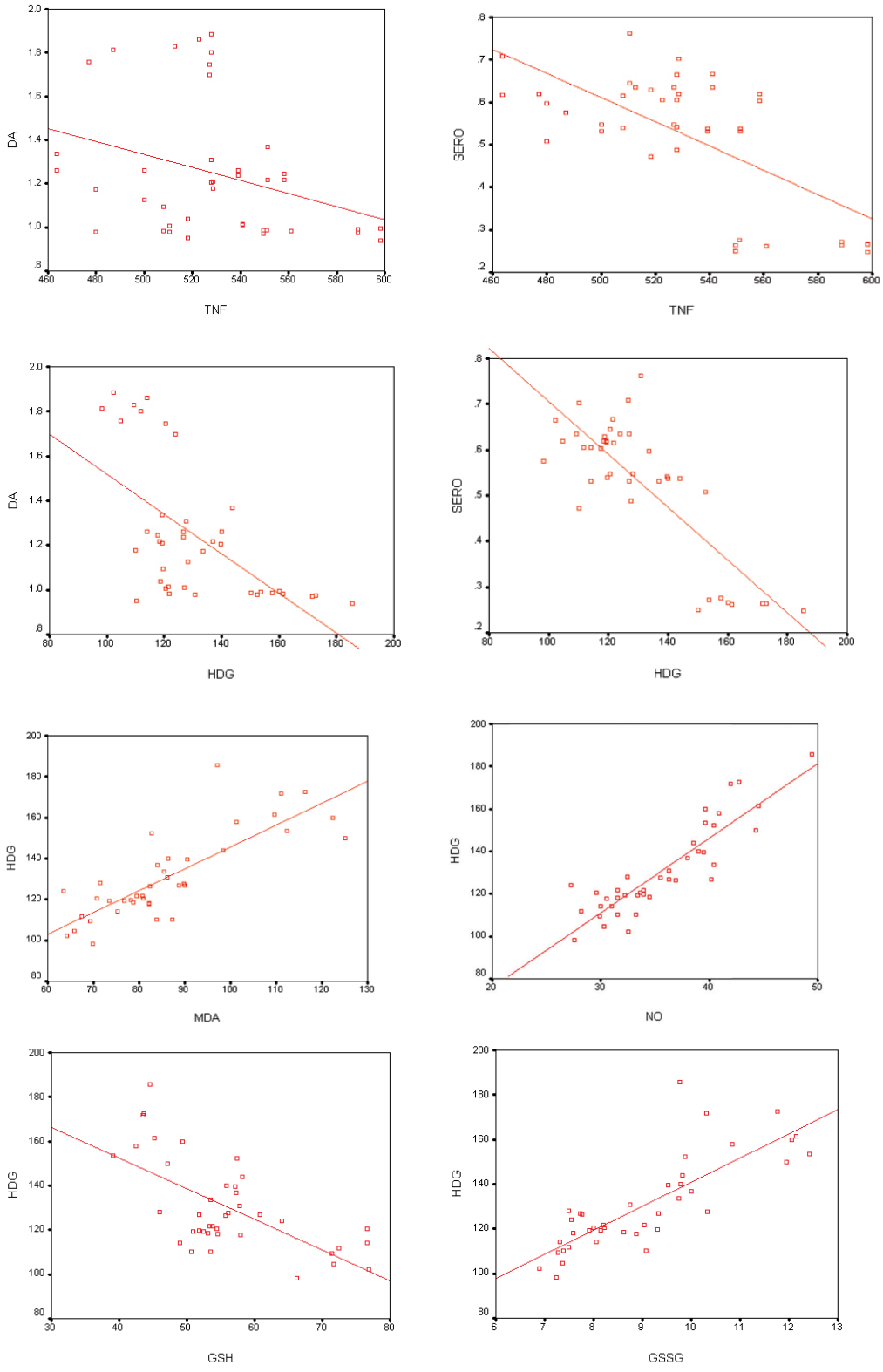


Figure 6. (Continued).



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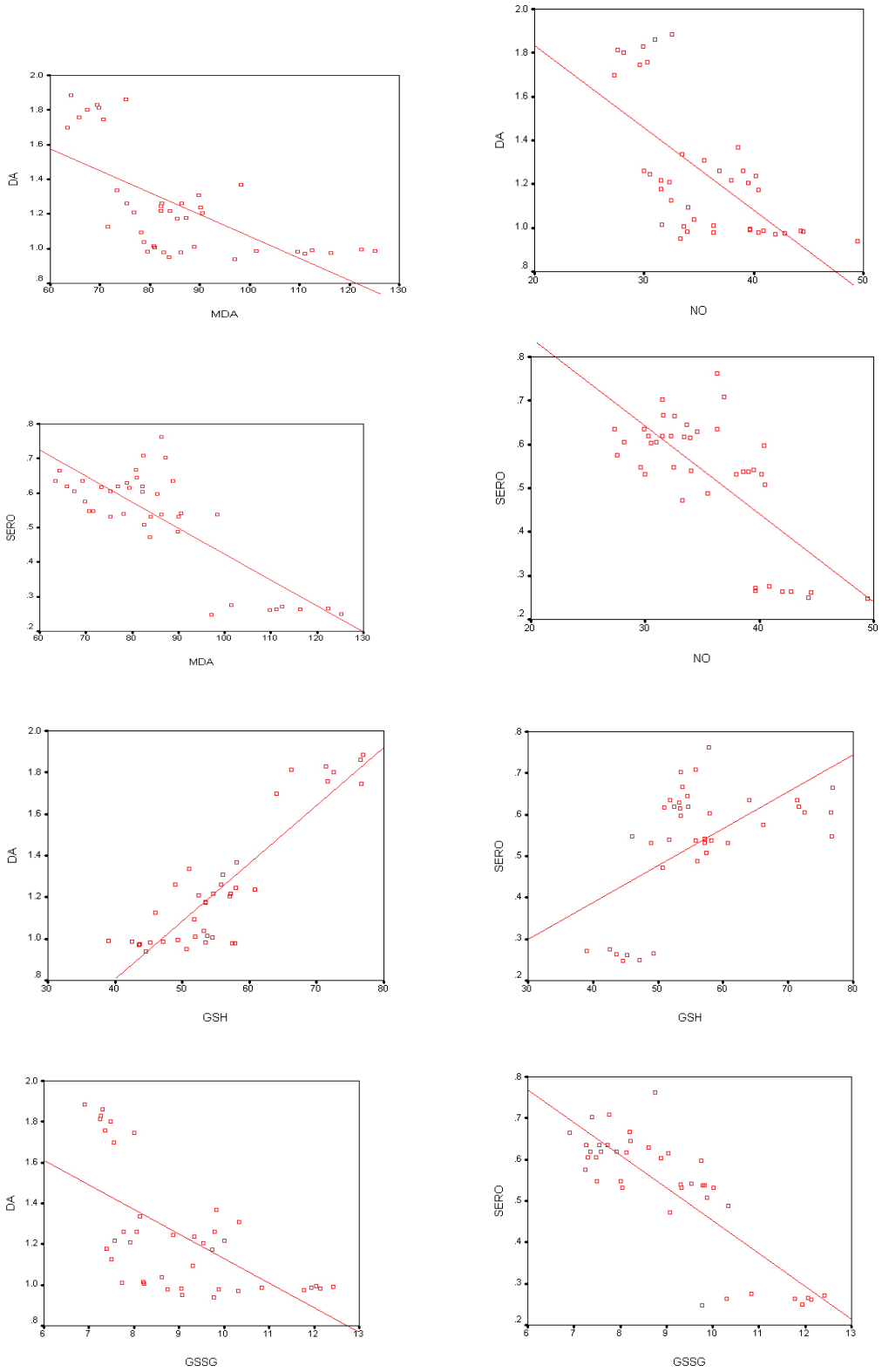

Figure 6. Correlation graphs.

DISCUSSION

As formerly reported; high fat in association with high fructose intake is linked to metabolic disturbances including IR, hypertriglyceridemia, dyslipidemia, in addition to hepatic steatosis. It was previously demonstrated that in dietary animal models of IR associated with very high-fat feeding; animals had significantly higher plasma insulin and marked increase in hepatic lipid levels compared to the control group fed with a standard diet.

Furthermore; it is well established that dietary intake high in fat and fructose triggers the explosion of free radicals and depletion of the antioxidant levels, thereby generating a redox imbalance, thus resulting in oxidative and nitrosative stresses in all body organs (Lozano *et al*., 2016; Rodriguez Lanzi *et al*., 2016). These resultant oxidative and nitrosative stresses accelerate oxidative DNA damage through amplification of lipid peroxidation in addition to the progression of structural and functional deformities in the cellular membrane as well as the intracellular biomolecules, organelles, and enzymes (D'Archivio *et al*., 2012; Fisher-Wellman *et al*., 2009). Formation of 8-hydroxy-2-deoxyguanosine (8-OHDG) adducts as a result of DNA oxidative hydroxylation could be a reliable oxidative DNA impairment and repair biomarker (Abdelali *et al*., 2016; Aksit *et al*., 2014).

As previously stated, depression is one of the most hazardous neurodegenerative disorders that has been implicated with IR as it could influence depression by several mechanisms, keeping in consideration the fact those variations in the activity of DA and/or serotonin systems have been correlated to depression; IR was previously proposed to disturb CNS serotonin (5-hydroxytryptamine; 5-HT) synthesis and decrease receptor affinity. On the other hand; IR has been reported to differ inversely with brain serotonergic activity. Moreover; multiple animal studies demonstrated metabolic improvements in IR models after the administration of DA agonists including significant weight loss, decreased levels of glucose along with increased glucose tolerance and decreased IR (Muldoon *et al*., 2006; Scranton and Cincotta, 2010). Furthermore; IR is accompanied with elevated inflammation and thereby cytokine outflow in some brain regions resulting in neuro-inflammation; a condition that could predispose depression by itself (Kleinridders *et al*., 2015; Wong *et al*., 2016). It was previously demonstrated that decreased depressive symptoms have been associated with inhibition of the inflammatory cytokine tumor necrosis factor (TNF-α) in depressed patients with elevated inflammation (Weinberger *et al*., 2015).

Metformin is a well-known anti-diabetic drug that is used in the management of IR. In the CNS, MT possesses antioxidant and neuroprotective activities and avoids the neurodegenerative alterations, thus the neuronal signaling system is restored (El-Mir *et al*., 2008).

Resveratrol is one of the most popular nutraceuticals that proved to have antioxidant, anti-inflammatory, antidepressant, and general neuroprotective properties (Ahmed *et al*., 2014b; Radwan *et al*., 2016). The neuroprotection of RSV on neural damages in various models has been well characterized. Several evidences have shown that RSV could exert a neuroprotective effect against ischemia, seizure, and many other neurodegenerative diseases. RSV ingestion in several stress and depression models reversed behavioral performance deficits and produced a marked increase of brain 5-HT, NE, and DA levels (Ahmed *et al*., 2014a). Moreover; increasing lines of evidence show that RSV exhibits cytoprotective actions and could influence DNA repair (Ahmed-Farid *et al*., 2016; Okawara *et al*., 2007). Increasing interest has focused on the possibility of the existence of a connection between RSV's anti-inflammatory and antioxidant potentials and neuroprotective properties. Previous investigators reported that RSV exhibited beneficial neuroprotective properties in several neurodegenerative models through up-regulating the antioxidant status, decreasing reactive oxygen species production, and attenuating the stimulation of immune cells and thus releasing of inflammatory mediators (Khan *et al*., 2010; Zhang *et al*., 2010). In addition; it was demonstrated that RSV improved insulin action in animals with experimentally induced IR. Furthermore; it showed advantageous actions against the neuro-complications associated with streptozotocin-induced diabetes in rats (Sadi and Konat, 2016; Szkudelski *et al*., 2015). Former studies in our laboratory revealed that the hepatic complications resulting from IR induced in rats by HFHF diet could be ameliorated by oral administration of RSV evidenced by the reduction in HOMA-IR, hepatic high affinity insulin receptors and low affinity insulin receptors expression, total cholesterol, triglycerides, TNF-α levels, as well as the serum aspartate aminotransferase and alanine aminotransferase. Furthermore, it counteracted the redox imbalance and exhibited hepatic DNA preservation potential (Saleh *et al*., 2017).

Therefore, the aim of our study was to investigate the possible neuroprotective role of RSV against the depression symptoms that could be associated with HFHF-induced IR compared to MT as a standard anti-diabetic drug with neuroprotective potential.

Correlation studies demonstrated that increased HOMA-IR, TNF-α, 8-OHDG levels, oxidative and nitrosative stresses were positively correlated. In addition, each one of these was individually negatively correlated to DA and serotonin levels.

Our study revealed that HFHF resulted in pronounced IR represented by significantly elevated HOMA-IR. Regarding the neuro-complications associated with IR in respect to animal behavior, our findings demonstrated that HFHF resulted in impairment in normal behavior designated by decreased ambulation and rearing frequencies in the OFT and increased immobility period in the FST. Concerning the biochemical parameters measured, there was an increase in monoamines turn over in addition to increased oxidative, nitrosative stresses, and TNF-α content as well as neuronal tissue degeneration represented by an elevated level of 8-OHDG.

Results of our study demonstrated that the ingestion of RSV improved the HFHF-induced insulin-resistant state where serum HOMA-IR was reduced. It reversed the impaired behavior in the OFT and decreased the immobility period in the FST. Moreover, brain monoamines turn over decreased; serotonin level was normalized, and the DA/NE ratio was shifted toward the DA side which could be responsible in part to the advantageous effect of RSV in ameliorating the insulin-resistant state. In addition, TNF-α level was reduced indicating the anti-inflammatory effect which in turn could be a contribution to the antidepressant mechanism of action. Finally, 8-OHDG content was lowered designating DNA preservation potential. The results were comparable to and sometimes superior over those of MT.

CONCLUSION

The present data indicate the antidepressant and neuro-preservation potential of RSV against the neuro-complications associated with HFHF-induced IR.

LIST OF ABBREVIATIONS

ALT = alanine aminotransferase, AST = aspartate aminotransferase, DA = dopamine, GSH = reduced glutathione, GSSG = oxidized glutathione, HAIR = high

affinity insulin receptors, HFHF = high fat high fructose, HOMA-IR = homeostatic model assessment of insulin resistance), HPLC = high-performance liquid chromatography, IR= insulin resistance, LAIR = low affinity insulin receptors, MDA= malondialdehyde, NE = norepinephrine, NOx = nitric oxide; nitrate+nitrite, ROS = reactive oxygen species, TNF-α = tumor necrosis factor-α, 5-HT = serotonin, 8-hydroxyguanosine= 8-OHDG.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

FINANCIAL SUPPORT

The authors declared that there are no sources of funding for the research.

AUTHORS' CONTRIBUTION

All authors contributed to the conception and design of the work. Dr. Rania F. Ahmed conducted behavioral studies. Biochemical parameters were conducted by Dr. Rania F. Ahmed, Dr. Sally A. El Awdan, Dr. Gehad A. Abdel Jaleel, Dr. Dalia O. Saleh, and Dr. Omar A.H. Ahmed-Farid. All authors contributed to the analysis and interpretation of data for the work, drafting the work, and revising it critically for important intellectual content.

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