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Effect of synthesized silver nanoparticles with *Rumex nervosus* leaves extract against obesity: Enzyme activity, DNA damage and gene expression profile of lipid pathway

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There are much more interest to develop novel anti-obesity agents extracted from herbal medicines. Therefore, this study investigated potential effect of *Rumex nervosus* leaves extract (RNLE) against obesity in rats exposed to high fat diet (HFD). Moreover, this study used RNLE-silver nanoparticles (RNLE-AgNPs) to increase the efficiency of its extract against obesity. Adult Wistar male rats were exposed to HFD for 12 weeks. Different concentrations of RNLE and RNLE-AgNPs were used to study activity and levels of ALT, AST, albumin, glucose, insulin and hepatocyte growth factor (HGF) and lipid profile as well as DNA damage and expression of lipid related genes (*Visfatin*, *HGF*, *Leptin*, *Glut*-1 and *Glut-4*). The results found that HFD rats exhibited significant high activities values of ALT and AST and low levels of albumin, high levels of LDL-C, HDL-C, TG and TC, high rate of DNA damage and significant expression alterations of lipid related genes compared to control rats. However, the best improvement of the previous negative findings was observed with almost all doses of RNLE-AgNPs compared to RNLE alone. The promising effects of RNLE-AgNPs might be attributed to the incidence of polyphenol compounds and flavanoids which might be responsible for antioxidant and anti-obesity capacity.

Keywords: ***Rumex nervosus,*** **Enzyme activity, Visfatin, DNA fragmentation, 8-OHdG generation, Gene expression, Obesity**

INTRODUCTION

Obesity is an abnormal condition in which body fat exceeded and accumulated to the level that it may cause an undesirable impact on health. Obesity is associated with critical health alterations such as diabetes, non-alcoholic fatty liver (NAFL) disease, hyperlipidemias and insulin resistance (Smith and Adams, 2011; Rodrigues et al., 2019). The imbalanced diet regime especially taking diet with high fat percentage is inducing NAFL disease. Moreover, taking high fat diet is inducing dysregulated pathway of many of biological actions such as expression of hepatic genes which affecting lipid oxidation and lipogenesis pathways (Jang et al., 2017).

Various genes are coinciding with lipid pathways that are taking part in a vital role within the regulation of lipogenesis in patients with NAFLD (Ozgocmen et al., 2018). One of the new prominent hormones regulating glucose levels is visfatin which is playing an important role in insulin pathway in the liver tissues (Memmert et al., 2019; Fukuhara et al., 2005; Adeghate, 2008). The expression of visfatin gene is over-expressed

by hyperglycaemia and hypoxia as well as inflammation. Hepatocyte growth factor (HGF) is growth regulator factor with strong mitogenic and angiogenic impacts (Nakagami et al., 2001; Morishita et al., 2002). Obesity is coincided with an elevation of over-expression of HGF gene and its secretion levels which associated with increase the body mass index (Rehman et al., 2003).

Growth regulators are important molecules associated with control body weight. One from the main regulators for body weight and adipose tissue mass is leptin which is enhanced and promoted by food intake. There is a correlation between leptin gene and leptin receptor gene and obesity (Paracchini et al., 2005). Glucose metabolism regulation in muscle and fat tissues is identified to be responsible for insulin sensitivity. In addition, uptake alteration of glucose by the cells and tissues is observed in obesity which causes insulin resistance (Marette et al., 1993; Brennan et al., 2004). This phenomenon is regulated by the expression profile of glucose transporter genes (GLUT1and GLUT4) in obese animals. Expression of GLUT1 and GLUT4 genes were decreased significantly in obese rats (Marette et al.,1993; Brennan et al., 2004).

Nowadays, there are much more interest to develop novel anti-obesity agents extracted from herbal medicines. This attention is much more raised due to the need to minimize the side effects related to currently available anti-obesity medications (Al-Naqeb and Taj Al-Deen, 2017). Numerous reports have been achieved on animals and clinical investigations using several medicinal herbs in which some studies verified considerable improvements in decrease body weight, hypoglycemia and hypolipidemia without any adverse impacts (Xiong et al., et al., 2010; Christine et al., 2011; Han et al., 2003). The attention to use such herbal plants due to presence of polyphenolic compounds in its extracts which play an important role as free radical scavenger against molecular alterations including DNA damage and expression changes of obesity related genes (Heyman-Lindén et al., 2016). The polyphenolic compounds have the ability to inhibit lipid peroxidation and expression alterations of adipose and hepatic tissues target genes (Heyman-Lindén et al., 2016; van Breda et al., 2018).

Rumex genus is one of the interesting medicinal plants due to its wide distribution in several Arabian countries including Saudi Arabia (Rechinger et al. 2001). Many of medicinal actions have been reported for traditional use of Rumex species. It has been used as anti-inflammatory, antibacterial, anti-cancer, anti-rheumatic, anthelmintic and hepatoprotective agents (Fatima et al., 2009; Humeera et al., 2013).

Use of nanomaterials in clinical and pharmaceutical applications has been reported to improve the drug efficiency due to its very low concentration, low side effects and low toxicity levels (Hegazy et al., 2018; Attallah et al., 2018; Abdu et al., 2017). Therefore, the main objective of this study was to use synthesized silver nano-materials with *R. nervosus* leaves extract against obesity in animal model.

MATERIALS AND METHODS

Sampling of plant material

Rumex nervosus plants were collected from the mountains in the south west namely Billasmar (18.7925° N, 42.2560° E), Saudi Arabian, on August 2018. *R. nervosus* leaves were identified and authenticated by a *Botanical taxonomy* expert at Faculty of Science, University of Tabuk, Kingdom of Saudi Arabia. The leaves were isolated from the plant branches, dried in open area, stored in plastic bags and kept at −20 ˚C, up to use.

Extract preparation

Powder of the dried samples (about 500 g) was extracted separately depending on the sample availability with alcohol (70% aqueous ethanol) in a volume of 500–700 ml. The samples were located in a shaker for seven days at 175 rpm at room temperature. After centrifugation the upper phase was separated and the residue was re-suspended in the same solvent for three times, then the centrifugation was done for the separated mixture again. The obtained supernatants were mixed and concentrated under reduced pressure at 40 ˚C till dryness to get EtOH extract of the *R. nervosus* leaves. The extract was kept in sterile tubes at 4ºC up to use.

Silver nanoparticles biosynthesis

The silver nitrate nanoparticles (1 mM) was synthesized in aqueous solution and added at a ratio of 9:1 with fresh *R. nervosus* leaves extract. This mixed solution was located with constant rotation in a shaker at room temperature (27 ± 2°C) for six h (Prasannaraj and Venkatachalam, 2017).

Characterization of *Rumex nervosus* leaves extract silver nanoparticles

In this study, nanoparticles classification using UV-visible spectrophotometry was implemented. So, UV-visible spectrophotometry (UVD 3200) had been used to prove the biosynthesize of *R. nervosus* leaves extract silver nanoparticles (RNLE-AgNPs). Also, X-ray *Diffractometer* (Equinox 3000) was used to perform the X-ray diffraction studies of RNLE-AgNPs. Moreover, to analyze the size and shape of the biosynthesized RNLE-AgNPs a Hitachi (S-4160) scanning electron microscopy was utilized. Nno-z 590 Malvern-Zetasizer was implemented to measure particle and molecule sizes of the prepared RNLE-AgNPs. The characterization assessment of RNLE-AgNPs was conducted to classify the functional groups responsible in reduction of silver ion existed in algae extract. Thus, potassium bromide with the pellets of algae extract combined with Ag-NPs (1:100) for FTIR spectrum analysis by a Bruker Tensor 27 spectrophotometer was used.

Experimental animals

Adult Wistar male rats (n=80, 150-160 g), were purchased from the Faculty of Science, University of Tabuk, Kingdom of Saudi Arabia, and were fed regular laboratory diet and water *ad libitum*. Following 7 days adaptation time, eight groups of animals were designed (10 rats each) in which the animals were housed separately in plastic cages. The animals were housed in contamination-free room which was controlled for temperature and light periods. The protocol of the study was applied depending on animal care guidelines in which the rats were not suffering at any time period of the experimentation according to the Medical Ethical Committee of Faculty of Science, University of Tabuk, Kingdom of Saudi Arabia.

Experimental protocol

The animals were allocated in 8 groups, ten animals each as follows: Group 1: Healthy control animals fed *ad-libitum*. Group 2: rats fed high fat diet *ad-libitum* for 12 weeks according to the method of Adaramoye *et al.,* (2008) and left untreated. Groups 3-5: animals fed high fat diet plus 0.05, 0.1 and 0.2 of *R. nervosus* leaves extract (RNLE), respectively for 12 weeks (Al-Naqeb and Taj Al-Deen, 2017). Groups 6-8: Animals fed *ad-libitum* with high fat diet plus 0.05, 0.1 and 0.2 of RNLE-AgNPs, respectively for 12 weeks (Table 1).

At termination of treatment, the rats were fasted overnight and the blood samples were aspirated under diethyl ether anaesthesia from the retro orbital venous plexus. Using cooling centrifuge all blood samples were centrifuged at 1800 g for ten min to obtain plasma which were stored at -20°C for biochemical analysis. After blood collection, all animals were rapidly sacrificed and the liver tissues were dissected, immediately frozen in liquid nitrogen and finally stored at -80 ºC for molecular biological analyses.

Biochemical analyses

Alanine transaminase and aspartate aminotransferase:

According to Reitman and Frankel (1957) serum ALT and AST activities were determined colorimetrically using Quimica Clinica Aplicada kit (Spain).

Albumin levels:

Levels of serum albumin were assessed using Stanbio Laboratory kit (Boerne, Texas, USA) according to Dumas and Biggs (1972) method.

Glucose levels:

According to Rinsler (1981) tools of plasma glucose were determined using Stanbio Laboratory kit (Boerne, Texas, USA).

Insulin levels:

ELISA (DRG kit Germany) assay was used to determine levels of insulin according to Temple et al., (1992) method.

Hepatocyte growth factor (HGF) levels:

Levels of HGF were measured using ELISA kit (Glory Science Co., Ltd, USA) according to Plum et al., (2009) method.

Lipid profile:

Low density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C), Triglyceride (TG) and total cholesterol (TC) were assessed using Randox assay kits (USA).

Comet Assay

The DNA damage in treated rat samples using comet assay was performed according to Blasiak et al., (2004). Homogenized liver samples of treated groups were mixed with low melting point agarose and loaded in small pieces on slides which pre-coated with normal melting

agarose. The loaded samples were kept on the slides in horizontal position for half hour in dark environment at 4°C. Low melting point agarose was then pipetting above the slides including samples and the slides were left for 30 min at 4°C to harden and put afterward in lysis buffer for one hour. Then, fresh alkaline unwinding buffer was used to submerge the slides in a dark place for one hour at 20°C. Afterwards, electrophoresis (0.8 V/cm, 300mAmps) for the slides were carried out for 30 min to assess the DNA damage in the form of tail migration in 100 cell per each animal. Specific software (TriTek corp., Comet Score, Sumerduck, VA22742) was used to determine the rate of the DNA damage per sample.

DNA fragmentation assay

Fragmentation of apoptotic DNA was carried out by detecting the laddering pattern according to Lu et al., (2002). After obtaining the isolated DNA, electrophoresis of the DNA was done on 1.5% agarose gel with ethidium bromide dye. A DNA ladder (Invitrogen, USA) was used as a molecular size marker and the fragments of the DNA were visualized by exposing the gels to UV transillumination.

DNA adducts (8-OHdG and 2-dG) determination

The generation ratio of 8-OHdG/2-dG in liver tissues of supplemented animals was assessed. The total DNA was extracted from animal samples by homogenizing the tissues in lysis buffer (pH 7.4), transferring and incubating them over night (~ for 16 h) with proteinase K at 50-60^{o}C. The incubated samples were treated with RNase enzyme for ten min at 50^{o}C according to Khalil et al., (2011). The method of chloroform/isoamyl alcohol was used to isolate the genomic DNA from treated rats. The extracted DNA content was dissolved in a solution of Tris/EDTA. The generation ratio of 8-OHdG/2-dG was assessed through several electrochemical sensors using HPLC (CoulArray system, Model 5600) (Girgis et al.,, 2012).

Expression analysis of lipid related genes

RNA isolation and reverse transcription reaction

TRIzol® extraction Chemical (Invitrogen) was utilized to isolate the total genomic RNA of adipose tissues of all treated animal. After completion of the isolation procedures, RNA pellet was stored in DEPC treated water. To digest the potential DNA residues the pellet of isolated RNA was treated with RNAse-free DNAse kit (Invitrogen, Germany). RNA aliquots were stored at -20°C or utilized immediately for reverse transcription (Salem et al., 2018).

First Strand cDNA Synthesis Kit (RevertAidTM, MBI Fermentas) was used to synthesize the cDNA copy from adipose tissues via reverse transcription reaction (RT). A RT reaction program of 25°C for 10 min, then one hour at 42 °C then 5 min at 95°C was used to obtain the cDNA copy of liver genome. Finally, tubes of reaction containing cDNA copy were collected on ice up to use for cDNA amplification (Khalil et al., 2018).

Table 1: Diet ingredient prepared for the experimental rats

Ingredient	Diet formulation of experimental rats (g/kg diet)							
	Normal Diet	HFD	HFD + RNLE (L)	HFD + RNLE (M)	HFD + RNLE (H)	HFD +RNLE-AgNPs (L)	HFD +RNL AgNPs (M)	HFD + RNLE AgNPs (H)
Casein	148	148	148	148	148	148	148	148
Sucrose	200	100	100	100	100	100	100	100
Starch	482	382	332	282	182	332	282	182
Cellulose	50	50	50	50	50	50	50	50
Fat	100	300	300	300	300	300	300	300
Vitamin Mix	10	10	10	10	10	10	10	10
Mineral Mix	10	10	10	10	10	10	10	10
RNLE	0	0	50	100	200	0	0	0
RNLE-AgNPs	0	0	0	0	0	50	100	200
Total	1000	1000	1000	1000	1000	1000	1000	1000

HFD: High fat diet

Table 2: Primers sequence used for qRT-PCR

Gene	Primer sequence	Reference
Visfatin	F: 5'-CCT TAC CTT AGA GTC ATT CA-3`	Assadi et al., (2015)
	R: 5'-GAC ATT CTC AAT ACT CCA C-3'	
HGF	F: 5'-AGC TCA GAA CCG ACC GGC TTG CAA CAG GAT-3'	Ahmed et al., (2015)
	R: 5`-TTA CCA ATG ATG CAA TTT CTA ATA TAG TCT-3`	
Leptin	F: 5`-GGT TCC TGT GGC TTT GGT CCT ATC T-3`	Roy et al., (2004)
	R: 5`-GTC TGG TCC ATC TTG GAC AAA CTC A-3`	
Glut-1	F: 5`-CTG TCG GGC ATC AAT GCT GTG T-3`	
	R: 5`-CCA AAG ATG GCC ACG ATA CTC AGA T-3`	
Glut-4	F: 5`-AAG AGA GCG TCC ACT GTC CTT GCT-3`	
	R: 5`-GAG ACC AAC GTG AAG ACG GTA TTG A-3`	
β-actin	F: 5`-GTG GGC CGC TCT AGG CAC CAA-3`	Khalil and Booles (2011)
	R: 5`-CTC TTT GAT GTC ACG CAC GAT TTC-3`	

F: forward primer; R: reverse primer. *HGF*: Hepatocyte growth facto; *Glut-1*: Glucose transporter 1; *Glut-4*: Glucose transporter 4

Quantitative Real Time-PCR

SYBR® Premix Ex TaqTM kit (TaKaRa, Biotech. Co. Ltd.) was used to perform the qRT-PCR analyses using the synthesized cDNA copies from adipose tissues. For each reaction a melting curve profile was conducted**.** The quantitative values of the target genes were normalized on the expression of the housekeeping gene (Table 2). The $2^{-\Delta\Delta CT}$ method was used to determine the quantitative values of the specific genes to the β-actin gene.

Statistical analysis

General Liner Models (GLM) of Statistical Analysis System (SAS) was used to analyses the data of biochemical parameters, DNA damage and gene expression assays. Afterwards, Scheffé-test was used to determine the significant differences between tested groups. The values are expressed as mean±SEM. All significance statements were based on probability of $P < 0.05$.

RESULTS

Effect of RNLE and RNLE-AgNPs on serum enzyme activities (ALT and AST) and protein (albumin) levels in obese rats

The results of serum ALT and AST activities as well as albumin levels in *obese* rats treated with RNLE and RNLE-AgNPs are illustrated in Table 3. The results found that rats fed high fat diet exhibited significant ($P<0.01$) high activities values of ALT and AST and low levels of albumin compared to control rats. In contrary, ALT and AST values and levels of albumin were improved with treatment of HFD-rats with RNLE and RNLE-AgNPs compared with those in rats fed HFD alone. The treatment of HFD-rats with *RNLE* decreased significantly ($P<0.05$) the ALT and AST values and increased levels of albumin with the high dose only, but this improvement was observed with RNLE-AgNPs at medium and high dose. Additionally, the best improvement of enzyme activities (ALT and AST) and protein (albumin) levels was observed at the high dose of RNLE-AgNPs.

Effect of RNLE and RNLE-AgNPs on glucose, insulin and HGF levels in obese rats

Table 4 shows levels of glucose, insulin and HGF in obese rats treated with RNLE and RNLE-AgNPs. The results revealed that rats fed high fat diet increased significantly ($P<0.01$) the levels of glucose, insulin and HGF compared to control rats. However, the levels of glucose, insulin and HGF were decreased with treatment of HFD-rats with RNLE and RNLE-AgNPs compared with those in rats fed high fat diet. The treatment of HFD-rats with *RNLE* decreased significantly ($P<0.05$) the levels of glucose, insulin and HGF with the high dose only, but this decrease was showed with RNLE-AgNPs at medium and high dose. Moreover, the lowest levels of levels of glucose, insulin and HGF were observed at the high dose of RNLE-AgNPs.

Effect of RNLE and RNLE-AgNPs on lipid profile in obese rats

Assessment of the lipid profile including TG, TC, LDL-C and HDL-C levels in obese rats treated with RNLE and RNLE-AgNPs is summarized in

Table 5. The results showed that feeding of rats with high fat diet increased significantly (P<0.01) the levels of TG, TC, LDL-C and HDL-C compared to control rats. In contrast, the levels of TG, TC, LDL-C and HDL-C were decreased with treatment of HFD-rats with RNLE and RNLE-AgNPs compared with those in rats fed HFD alone. The treatment of HFD-rats with *RNLE* decreased significantly (P<0.01) the levels of TG and TC with the medium and high dose only, but this decrease was showed with RNLE-AgNPs at all three doses. The levels of LDL-C were decreased significantly (P<0.05) in HFD-rats treated with RNLE at the high dose only, but this decrease was showed with RNLE-AgNPs at medium and high dose. The levels of HDL-C were decreased significantly (P<0.05) in HFD-rats treated with RNLE-AgNPs at high dose only.

Effect of RNLE and RNLE-AgNPs on DNA damage in obese rats

Table 6 shows rates of DNA damage in liver tissues of obese rats treated with RNLE and RNLE-AgNPs. The results showed that feeding of rats with high fat diet elevated considerably (P<0.01) the DNA damage mean values compared to control rats. However, the levels of DNA damage were decreased significantly (P<0.01) with treatment of HFD-rats with RNLE (at high dose) and RNLE-AgNPs (at medium and high dose) compared with those in rats fed HFD alone.

Effect of RNLE and RNLE-AgNPs on DNA fragmentation in obese rats

Figure 1 represents DNA fragmentation in liver tissues of obese rats treated with RNLE and RNLE-AgNPs. The results showed that rats fed high fat diet exhibited high fragmented DNA bands compared to control rats. However, the fragmented DNA bands were decreased in HFD-rats treated with RNLE-AgNPs much more than *RNLE*. The DNA fragmentation in rats treated with medium and high dose of RNLE-AgNPs was relatively close to that in control rats.

Effect of RNLE and RNLE-AgNPs on 8-OHdG generation in obese rats

The 8-OHdG/2-dG generation ratio in liver tissues of obese rats treated with RNLE and R. nervosus nanoparticles is summarized in Figure 2. The results found feeding of male rats with HFD increased significantly (*P*<0.01) the OHdG/2-dG generation rate compared with control rats. On the other hand, the generation ratio of 8-OHdG/2-dG decreased significantly (*P*<0.05) in HFD-rats treated with medium and high dose of RNLE and RNLE-AgNPs compared with rats fed HFD alone. Additionally, the OHdG/2-dG generation rate in HFD-rats treated with high dose RNLE-AgNPs was decreased to a level close relatively to that in control rats.

Table 3: Effect of *R. nervosus* extract and *R. nervosus* nanoparticles on serum activities of ALT & AST and levels of albumin in obese rats.

Treatment	ALT (U/L)	AST (U/L)	Albumin (g/dl)
Control	28.43±2.1^{c}	23.74±1.3^{d}	5.18±0.04^{a}
HFD	62.94±2.8^{a}	84.55±4.2^{a}	2.16±0.02^{c}
HFD + RNLE (L)	56.31±2.3^{ab}	79.42±3.6^{a}	2.78±0.05^{bc}
HFD + RNLE (M)	51.72±1.4^{ab}	68.49±1.7^{ab}	2.94±0.03^{bc}
HFD + RNLE (H)	44.52±1.6^{b}	56.84±1.8^{b}	3.54±0.06^{b}
HFD + RNLE-AgNPs (L)	54.86±1.8^{ab}	74.52±2.5^{ab}	3.11±0.01^{b}
HFD + RNLE-AgNPs (M)	42.75±2.2^{b}	55.68±1.4^{b}	3.67±0.02^{ab}
HFD + RNLE-AgNPs (H)	35.82±1.5^{c}	43.39±2.2^{c}	4.52±0.07^{a}

HFD: High fat diet; RNLE: *Rumex nervosus* leaves extract; AgNPs: Silver nanoparticles; (L): Low dose; (M): Medium dose; (H): High dose; Data are presented as mean ± SEM. ^{a,b,c,d} Mean values within tissue with unlike superscript letters were significantly different (*P*< 0.05, Scheffé-Test).

Table 4: Effect of *R. nervosus* extract and *R. nervosus* nanoparticles on glucose, insulin and HGF levels in obese rats.

Treatment	Glucose (mg/dl)	Insulin mU/ml	HGF (ng/L)
Control	89.24±3.2^{c}	13.41 ±0.6^{c}	106.28 ±3.4^{d}
HFD	156.17±4.6^{a}	23.52 ±1.1^{a}	203.14 ±8.2^{a}
HFD + RNLE (L)	146.54±5.1^{a}	21.94 ±0.9^{a}	196.38 ±4.1^{a}
HFD + RNLE (M)	138.61±2.5^{ab}	20.78 ±0.8^{ab}	187.46±3.2^{ab}
HFD + RNLE (H)	126.12±1.7^{b}	17.81 ±1.2^{b}	153.87±2.7^{b}
HFD + RNLE-AgNPs (L)	132.52±3.3^{ab}	21.35 ±0.7^{ab}	185.24±1.6^{ab}
HFD + RNLE-AgNPs (M)	115.81±4.1^{b}	18.55 ±0.5^{b}	141.19±2.2^{b}
HFD + RNLE-AgNPs (H)	97.34±2.8^{c}	15.96 ±0.4^{bc}	128.55±2.5^{c}

HGF: Hepatocyte growth factor; HFD: High fat diet; RNLE: *Rumex nervosus* leaves extract; AgNPs: Silver nanoparticles; (L): Low dose; (M): Medium dose; (H): High dose; Data are presented as mean ± SEM. ^{a,b,c,d} Mean values within tissue with unlike superscript letters were significantly different (P< 0.05, Scheffé-Test).

Table 5: Effect of *R. nervosus* extract and *R. nervosus* nanoparticles on lipid profile (TG, TC, LDL-C and HDL-C) in obese rats.

Treatment	TG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
Control	58.27±4.7^{e}	64.31±1.7^{c}	12.61±0.08^{c}	51.42±1.1^{b}
HFD	261.46±5.1^{a}	102.75±2.9^{a}	20.11±1.02^{a}	62.88±3.2^{a}
HFD + RNLE (L)	243.42±6.3^{ab}	92.65±3.6^{ab}	19.45±0.06^{a}	61.25±1.5^{a}
HFD + RNLE (M)	237.22±4.8^{b}	84.23±2.4^{b}	18.97±1.03^{ab}	59.33±2.8^{a}
HFD + RNLE (H)	208.51±5.3^{c}	81.72±4.1^{b}	16.14±0.09^{b}	56.95±2.2^{ab}
HFD + RNLE-AgNPs (L)	226.92±6.7^{b}	83.25±2.3^{b}	18.23±0.06^{ab}	58.21±3.1^{ab}
HFD + RNLE-AgNPs (M)	201.74±3.9^{c}	73.44±2.8^{bc}	16.95±0.08^{b}	55.19±2.4^{ab}
HFD + RNLE-AgNPs (H)	173.62±4.5^{d}	66.19±1.2^{c}	13.82±0.05^{bc}	52.65±3.6^{b}

TG: Triglyceride; TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High-density lipoprotein-cholesterol; HFD: High fat diet; RNLE: *Rumex nervosus* leaves extract; AgNPs: Silver nanoparticles; (L): Low dose; (M): Medium dose; (H): High dose; Data are presented as mean ± SEM. ^{a,b,c,d,e} Mean values within tissue with unlike superscript letters were significantly different (P< 0.05, Scheffé-Test).

Table 6: Effect of *R. nervosus* extract and *R. nervosus* nanoparticles on rate of DNA damage in liver tissues of obese rats.

Treatment	No. of cells		Class[¥] of comet				DNA damaged cells (mean ± SEM)
	Analyzed	Total comets	0	1	2	3	
Control	500	29	471	26	3	0	5.8±0.11^{c}
HFD	500	88	412	35	27	26	17.6±0.62^{a}
HFD + RNLE (L)	500	79	421	34	25	20	15.8±0.52^{ab}
HFD + RNLE (M)	500	77	423	32	34	11	15.4±0.41^{ab}
HFD + RNLE (H)	500	64	436	29	23	12	12.8±0.27^{b}
HFD + RNLE-AgNPs (L)	500	67	433	28	21	18	13.4±0.39^{b}
HFD + RNLE-AgNPs (M)	500	53	447	26	16	11	10.6±0.18^{bc}
HFD + RNLE-AgNPs (H)	500	39	461	21	17	1	7.8±0.09^{c}

[¥]: Class 0= no tail; 1= tail length < diameter of nucleus; 2= tail length between 1X and 2X the diameter of nucleus; and 3= tail length > 2X the diameter of nucleus.(*): No of cells analyzed were 100 per an animal. HGF: Hepatocyte growth factor; HFD: High fat diet; RNLE: *Rumex nervosus* leaves extract; AgNPs: Silver nanoparticles; (L): Low dose; (M): Medium dose; (H): High dose; Data are presented as mean ± SEM. ^{a,b,c} Mean values within tissue with unlike superscript letters were significantly different (P< 0.05, Scheffé-Test).

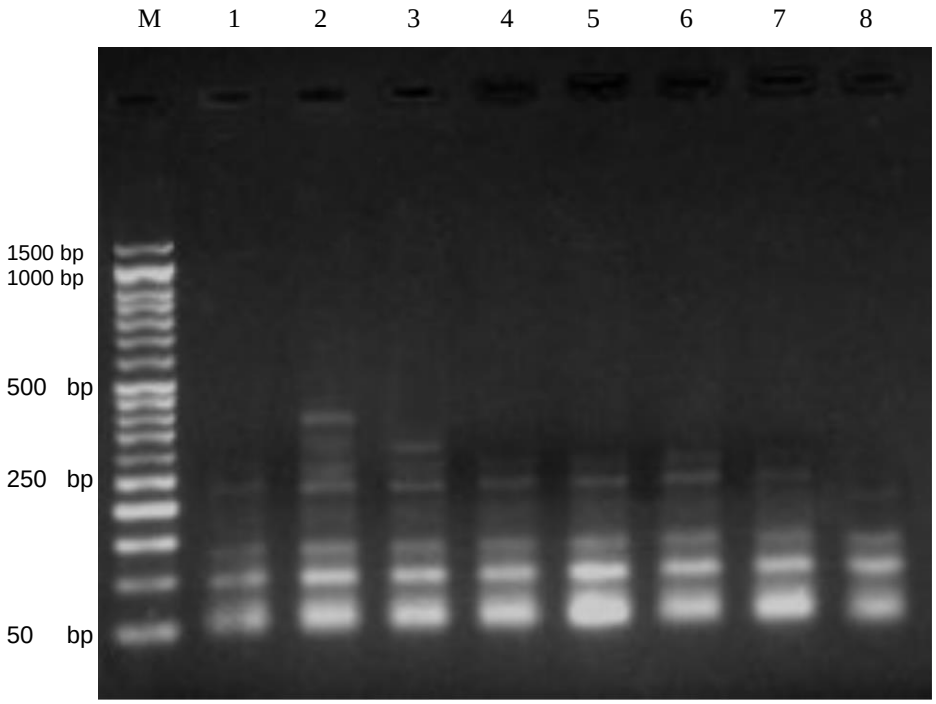

Figure 1: DNA fragmentation detected with agarose gel in liver tissues of obese rats treated with RNLE and R. nervosus nanoparticles.

M: represents DNA marker, Lane 1: represents control rats; Lane 2: represents obese rats; Lanes (3-5): represent obese rats treated with low, medium and high doses of RNLE, respectively; Lanes (6-8): represent obese rats treated with low, medium and high doses of RNLE-AgNPs, respectively

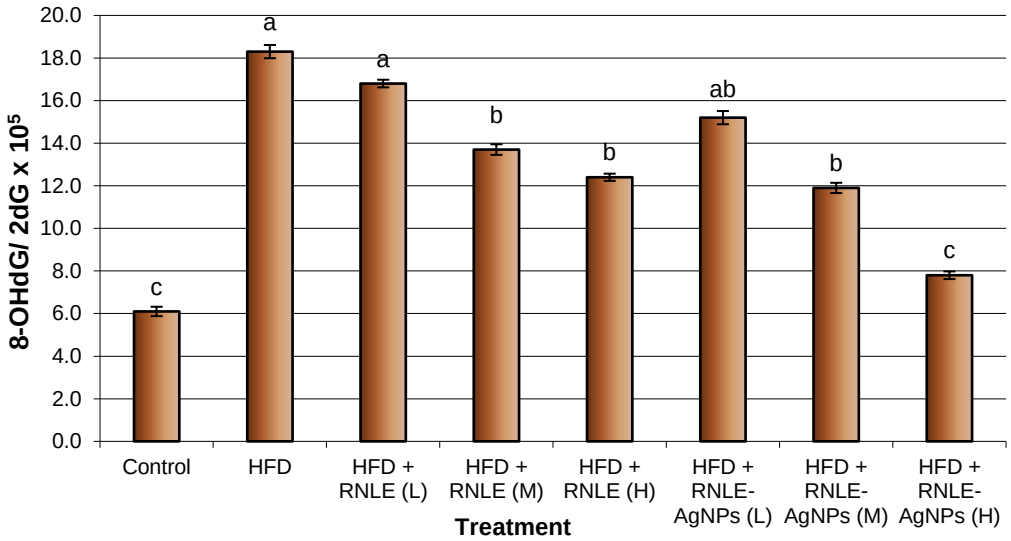

Figure 2: Ratio of DNA adducts (8-OHdG/2dG) in liver tissues of obese rats treated with *RNLE* and *R. nervosus* nanoparticles.

Results are expressed as mean±SEM. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (*P*< 0.05, Scheffé-Test).

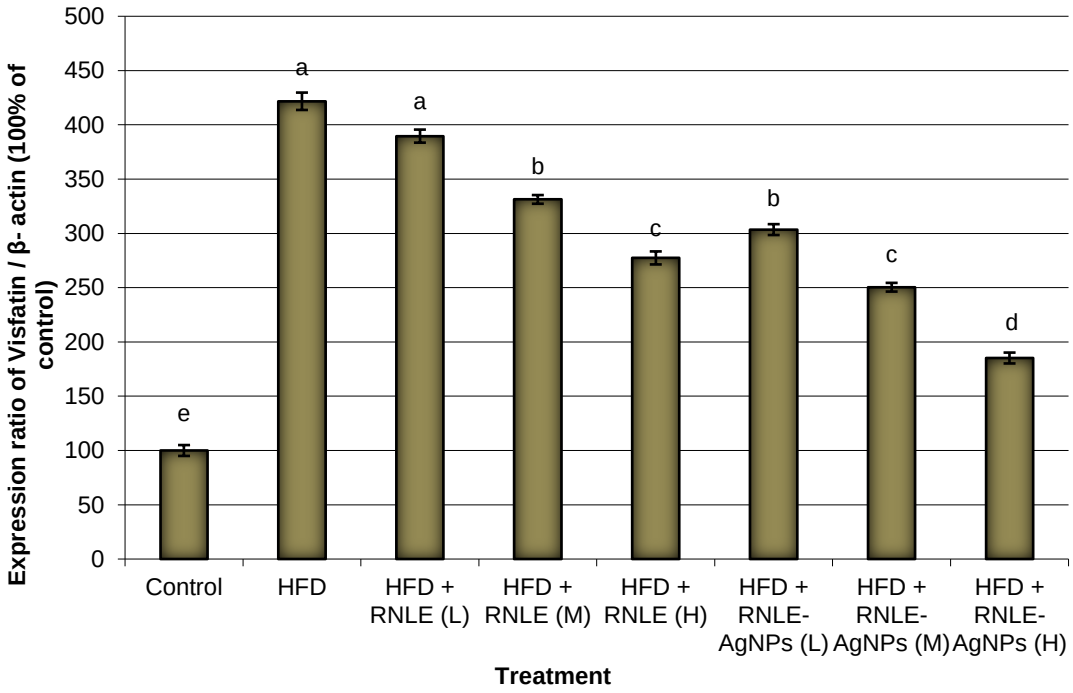

Figure 3: Expression change of *Visfatin* gene in adipose tissues.
Data are presented as mean ± SEM. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (*P*< 0.05, Scheffé-Test).

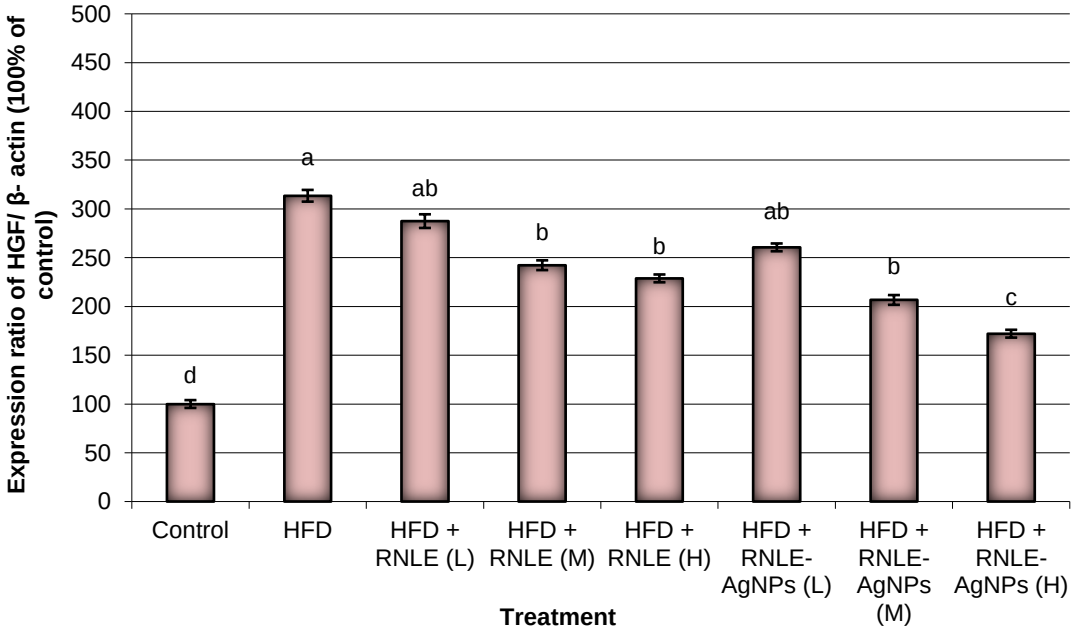

Figure 4: Expression change of *HGF* gene in adipose tissues.
Data are presented as mean ± SEM. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (*P*< 0.05, Scheffé-Test).

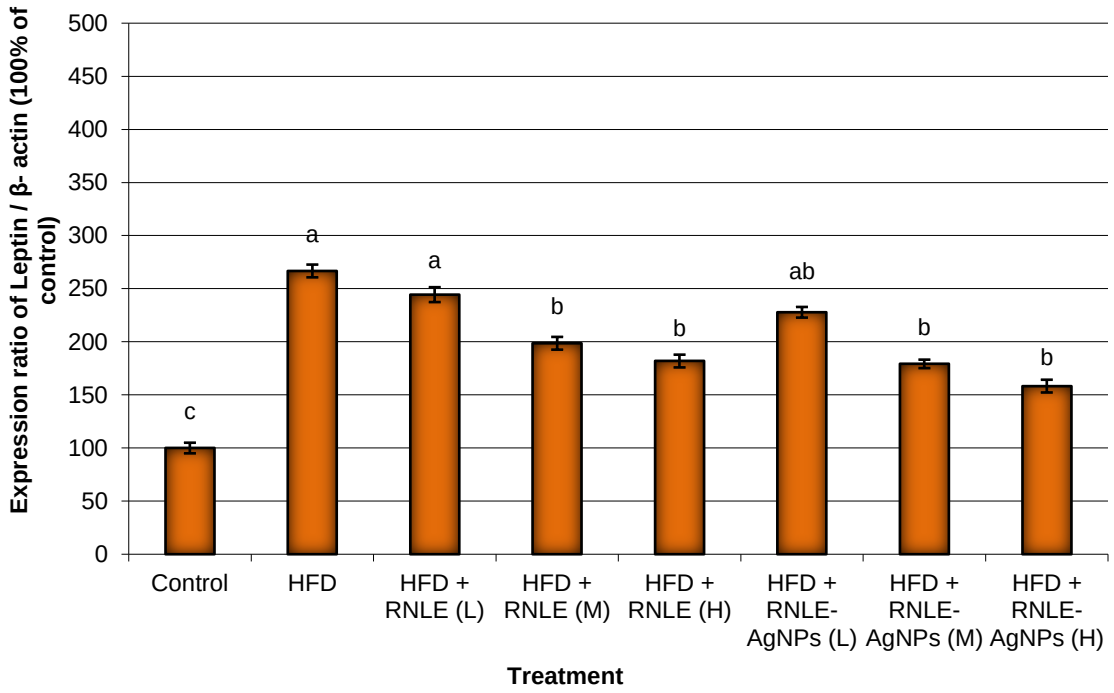

Figure 5: Expression change of *Leptin* gene in adipose tissues.
Data are presented as mean ± SEM. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (*P*< 0.05, Scheffé-Test).

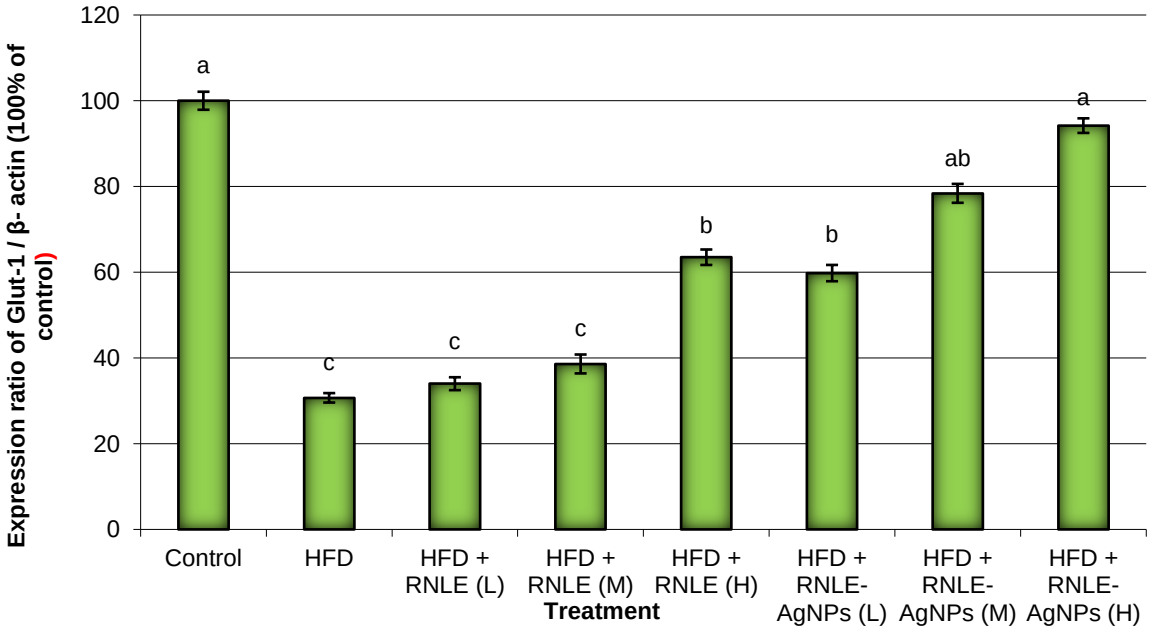

Figure 6: Expression change of *Glut-1* gene in adipose tissues.
Data are presented as mean ± SEM. [a,b,c] Mean values within tissue with unlike superscript letters were significantly different (*P*< 0.05, Scheffé-Test).

Effect of RNLE and RNLE-AgNPs on expression of lipid related genes

The expression of lipid related genes (*Visfatin*, *HGF*, *Leptin*, *Glut*-1 and *Glut-4*) in liver tissues of obese rats treated with RNLE and RNLE-AgNPs is summarized in Figures 3-7.

The results found that the expression levels of *Visfatin*, *HGF* and *Leptin* genes in male rats fed HFD were up-regulated significantly (P<0.01) compared with control rats. In contrast, the expression levels of *Visfatin*, *HGF* and *Leptin* genes in HFD-rats treated with RNLE and RNLE-AgNPs (at medium and high dose) were down-regulated significantly compared with rats fed HFD. Moreover, HFD-rats treated with low dose of RNLE-AgNPs exhibited significant decrease in the expression levels of *Visfatin* gene compared with rats fed HFD alone.

On the other hand, the expression levels of *Glut-1 and Glut-4* genes in male rats fed HFD were decreased significantly (P<0.01) compared with control rats. In contrast, the expression levels of *Glut-1* gene was increased significantly in HFD-rats treated with high dose of RNLE compared with rats fed HFD. Furthermore, the expression levels of *Glut-4* gene was down-regulated (P<0.51) in HFD-rats treated with medium and high dose of *RNLE* compared with rats fed HFD. Moreover, the expression levels of *Glut-1* and *Glut-4* gene were highly (P<0.51) down-regulated in HFD-rats treated with all doses of RNLE-AgNPs compared with rats fed HFD

DISCUSSION

Several studies reported that feed on diet containing high fat is associated with increase body mass index which can induce obesity and other related diseases. The present study also found that feeding of rats on HFD for three months increased the body weight and other biochemical related parameters verifying the obese disease (Neyrinck et al., 2009).

The present study proved that rats fed high fat diet exhibited significant high activities values of ALT and AST and low levels of albumin compared to control rats. These findings are in same line with Hooper et al., (2011). They reported that activities of ALT and AST are increased significantly in liver tissues of patient which reflecting hepatocyte damage status (Yang et al., 2009). It has been reported that increase the aminotransferases activities in liver indicating elevation of oxidative stress status which could enhance: (a) acute oxidative change in liver tissues inducing injury cell viability and loss of cell function; (b) expression alteration of oxidative related genes (Videla, 2009; Jacobs et al., 2011).

The results of this study proved that levels of albumin in HFD group were significantly reduced. These findings are in agreement with Ahmed et al. (2015) and Farrell and Larter (2006), in which the levels of albumin were markedly decreased in fatty liver diseases. Albumin protein is mostly synthesized in liver which binds with many of ligands. So, oxidative stress in liver tissues might cause alteration in serum albumin and therefore affecting the binding properties with ligands (Amirtharaj et al., 2008). Hence, the oxidative stress in liver tissues could be the main reason of albumin reduction in HFD group. Another fact that oxidative stress in fatty liver increases the free fatty acids and consequently increase levels of ROS in liver tissues (Lewis et al., 2002).

The present study proved that ALT and AST values and levels of albumin were improved with treatment of HFD-rats especially with RNLE-AgNPs compared with those in rats fed HFD alone. Desta et al., (2006) reported that *R. nervosus* have 18 polyphenol compounds which existing in leaves. So, the antioxidant properties and free radical scavenging activity could be attributed to the polyphenol compounds existing in *R. nervosus* leaves extract. Desta et al., (2006) reported that four polyphenol compounds (catechin, chlorogenic acid, orientin, and apigenin-O-acetylglycoside) in *R. nervosus* have strong antioxidant activity could be responsible for free radical scavenging activity in liver tissues.

The current study exhibited that HFD group of rats indicated significant increase in the levels of glucose and insulin levels compared to control rats. The results reported by Yun et al., (2009) and Ahmed et al., (2015) agree the current findings. The fact on increase glucose levels in obese animal is that the presences of high levels of lipolytic activity as a result of fat accumulation producing high levels of free fatty acids to liver tissues. Thus, the increase level of fatty acids transferred to liver accelerates gluconeogenesis and consequently glucose levels (Giannini et al., 2005; Ginsberg and Stalenhoef, 2003). Additionally, accumulation of fat in HFD rats affects pancreatic β-cells under high-glucose conditions preventing the role glucose transporter-4 (GLUT) in transfer glucose to muscle increasing glucose and insulin levels as well as HGF in which the later is strongly correlated with increase of insulin levels (Jung and Kang, 2010; Wilcox, 2005).

The levels of glucose and insulin as well as HGF levels in the current study were decreased significantly by treatment of HFD-rats with RNLE and RNLE-AgNPs. The antioxidant agents existing in RNLE might be responsible to decreasing the glucose, insulin and HGF levels (Ahmad et al., 2019). The polyphenol compounds have been reported to restore the function of pancreatic β-cell and then decrease glucose level (Ahmad et al., 2019; Desta et al., 2006).

The current study revealed that lipid profile including TG, TC, LDL-C and HDL-C was increased in rats exposed to HFD compared to control rats. However, these levels of TG, TC, LDL-C and HDL-C were decreased with treatment of HFD-rats with RNLE and RNLE-AgNPs. In the same line, Al-Naqeb and Taj Al-Deen (2017) reported that *RNLE* showed presence of three flavanoids; isohamnetin, quercetin and kaempferol which might be responsible for reducing levels of TG, TC, LDL-C and HDL-C in obese rats. Moreover, the high levels of antioxidants in *RNLE* can reduce lipid parameters by trapping lipid peroxyl, peroxides and hydroperoxides which lead to repair the lipid profile (Hu and Willett, 2002; Valko et al., 2007).

The results of this study found that HFD group of rats exhibited high levels of the DNA damage, DNA fragmentation and DNA adducts compared to control rats. In contrast, the levels of DNA damage, DNA fragmentation and DNA adducts were decreased significantly in HFD-rats treated with RNLE and RNLE-AgNPs. Oxidative stress in liver tissues due to accumulation of fat and generation of free fatty acids might be main reason for generation of ROS levels in liver and consequently induce DNA damage (Valko et al., 2007). On the other hand, presence of phytochemical and antioxidants in *RNLE* modulate hepatic pathway to prevent the DNA damage in HFD-rats treated with *R. nervosus* (Nunez-Selles, 2005; Chen et al., 2013; Araya et al., 2015).

Expression of lipid profile related genes exhibited that expression levels of *Visfatin*, *HGF* and *Leptin* genes in male rats fed HFD were up-regulated significantly compared with control rats. Our findings are consistent with numerous studies revealed that there are strong correlation between over-expression of *Visfatin*, *HGF* and *Leptin* genes and obesity and accumulation of fats (Rehman et al., 2003; Paracchini et al., 2005; Haider et al., 2006; Saddi-Rosa et al., 2010). Regulation glucose metabolism in muscle and fat tissues is identified to be responsible for insulin sensitivity. Visfatin is increased by adipose tissues and play main role in glucose and insulin secretion (Assadi et al., 2015). Rehman et al. (2003) reported that the increase the expression levels of HGF gene in obese patients is strong correlated with obesity rather than other diseases such as diabetes or hypertension. Paracchini et al., (2005) suggested that leptin gene is one of the main genes in obesity pathway. Its expression is normally increased by increase adipose tissue size and free fatty acids (Maffei et al., 1995).

The current study found that the expression levels of *Visfatin*, *HGF* and *Leptin* genes in HFD-rats treated with RNLE and RNLE-AgNPs were down-regulated significantly compared with rats fed HFD. Chuang et al., (2010) suggested that flavonoids existed in R. nervosus play a vital role in down regulation of lipid regulated genes. Additionally, the flavanoids isohamnetin, quercetin and kaempferol might be responsible for reducing levels of Visfatin, HGF and Leptin genes in obese rats. Furthermore, the high levels of antioxidants in *RNLE* could play regulation role in the expression of lipid related genes (Derdemezis et al., 2011).

It has been reported that expression of GLUT1 and GLUT4 genes is negatively correlated with the insulin levels. The expression levels of GLUT1 and GLUT4 were decreased significantly in obese rats (Marette et al., 1993; Brennan et al., 2004). This fact was observed in the results of the present study in male rats fed HFD. In contrary, the expression levels of GLUT1 and GLUT4 genes in HFD-rats treated with RNLE and RNLE-AgNPs were increased significantly compared with obese rats. The expression of GLUT1 and GLUT4 genes is correlated with regulation of glucose metabolism which is markedly improved by the active ingredients in *R. nervosus*.

CONCLUSION

In conclusion, *R. nervosus* exhibited promising effects of improving enzyme activity, preventing DNA damage and decreased gene expression alterations in obese rats. These actions were much more effective in *R. nervosus* nanoparticle than *R. nervosus* extract. The promising effects of *R. nervosus* nanoparticle might be attributed to the presence of polyphenol compounds (catechin, chlorogenic acid, orientin, and apigenin- O-acetylglycoside) and flavanoids (isohamnetin, quercetin and kaempferol) which could be responsible for antioxidant and anti-obesity capacity.

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CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

AA collected the plant samples, prepared NPs, designed and performleed the experiments. AA performed animal treatments, tissue collection, carried out the biochemical (ALT, AST, insulin, albumin, LDL-C, HDL-C, TG and TC) and genetic analyses (Comet, DNA fragmentation, DNA adducts and gene expression assay) and data analysis. AA wrote and submitted the manuscript. AA reviewed the manuscript in final version. AA read and approved the final version.

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