Effect of Exercise and Quercetin on Obesity Induced Metabolic and Renal Impairments in Albino Rats

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Abstract

Obesity has been described as a state of chronic oxidative stress and inflammatory reaction. This study was carried out to clarify the effect of exercise and antioxidant quercetin on oxidative stress and inflammatory reaction induced by obesity in renal tissue. Rats were randomly divided into five groups 8 animals each: Group I, received balanced diet; Group II, received high fat diet; Group III, received high fat diet and were subjected to exercise training for the last 8 weeks; Group IV, received high fat diet and treated with quercetin at a dose of 15gm/kg intraperitoneal (IP) daily in the last 8 weeks; Group V, received high fat diet and were subjected to exercise training combined with quercetin treatment in the last 8 weeks. The study was carried out over 28 weeks. Obesity induced by high fat diet (HFD) was confirmed by measuring body weight and body mass index (BMI). Obese rats developed insulin resistance confirmed biochemically by significant increase in fasting blood glucose, fasting insulin and homeostasis model assessment of insulin resistance (HOMA-IR) index. Moreover, there was significant increase in serum urea, serum creatinine, albuminurea, renal malondialdehyde (MDA) and tumor necrosis factor alpha (TNF-α) levels with significant decrease in superoxide dismutase (SOD), reduced glutathione (GSH) and adiponectin. Both exercise training and quercetin treatment were sufficient to cause improvement in insulin resistance, kidney functions and renal oxidative stress, with significant decrease in TNF-α and increase in adiponectin. The most improvement was among combined exercise & antioxidant group. We concluded that anti-oxidative and anti-inflammatory effect of exercise & quercetin may be through modulation of adiponectin. Moreover, histopathological examination showed mild improvement of HFD induced lesion of renal tissue in exercise and quercetin groups with more superior effects of their combination.

Keywords: Obesity, oxidative stress, exercise, antioxidants, quercetin, adiponectin.

Introduction

Obesity is a chronic disease of multifactorial origin and can be defined as an increase in the accumulation of body fat. It seems to be a condition in which kidneys demonstrate morphological and functional alterations. Adipose tissue is a triglyceride storage organ, but studies have also shown the role of white adipose tissue as a producer of certain bioactive substances called adipokines. These adipokines induce the production of reactive oxygen species (ROS), generating a state of systemic oxidative stress (Fernández et al., 2011). ROS play a crucial role in mediating renal injury (Jaimes et al., 2010). Higher production of ROS results in the modification of proteins and lipids, the activation of several stress-induced transcription factors, and the production of proinflammatory and anti-inflammatory cytokines. The mechanisms of this renal inflammation are still unclear (Scandalios 2004). The kidney is an organ highly liable to damage caused by ROS, likely due to the abundance of long-chain polyunsaturated fatty acids in the composition of renal lipids (Noeman et al., 2011). ROS are highly reactive molecules that oxidize lipids and proteins, cause cellular injury, and promote glomerular and renal tubule injury and associated proteinuria (Habibi et al., 2011).

There is evidence that exercise prevents oxidative damage by reducing oxidative stress (Schiffrin and Touyz 2004). Exercise appears to act as a natural antioxidant and anti-inflammatory strategy for preventing obesity-associated complications. It improves glucose-insulin homeostasis and antioxidant defenses (Savini et al., 2013). Quercetin is one of the most widely distributed flavonoids and it has been shown to reduce oxidative stress via their antioxidant effects in protecting cellular components against ROS (Khaki et al., 2009). It has been demonstrated that quercetin exhibits its therapeutic potential against many diseases, including ischemic heart diseases, atherosclerosis, liver fibrosis, renal injury, and chronic biliary obstruction (Tieppo et al., 2007). Excessive caloric intake contributes to adiposity and initiates a cascade that ultimately leads to end-organ dysfunction including obesity-related chronic renal disease. Recent studies demonstrate that adiponectin are key proteins orchestrating organ crosstalk between fat cells and the kidney (Tsuda et al., 2008; Sharma et al., 2008). Because adiponectin levels are secreted from adipocytes and related inversely to the amount of adiposity, these data identify adiponectin as a candidate mediator of adipose and kidney crosstalk. Many studies report conflicting data between adiponectin levels and mortality in patients with chronic renal disease (Sharma et al., 2008).

It is important to study new strategies to prevent the growth of obesity and related diseases which is expected in the coming years. Exercise seems to be a powerful tool to face obesity problems. So this study was undertaken to investigate the preventive role of the swimming exercise and quercetin against the renal oxidative stress and inflammatory reaction that develops in obesity. And to investigate whether swimming exercise is superior to quercetin as a preventive measure for renal dysfunction in obesity. We also aimed at detecting the role of adiponectin in obesity induced renal dysfunction.

Materials and Methods

Experimental Animals

All of the animals were approved by the Ethical Committee of the Faculty of Medicine, Benha University, Egypt. This study was conducted on 40, 6-8 weeks old, adult Wistar albino male rats each weighing between 170 and 200 gm. Animals were housed in the animal laboratory at the medical research center at Benha faculty of medicine.

Groups of the Experiment

The animals were randomly divided into 5 groups, each consisting of 8 rats. The groups are as follows:

- **Group I (Control Group)**
  Received a balanced diet in which fat represented 12% of total caloric intake for 24 weeks.
Group II (Obesity Group)
Received a high fat diet in which fat represented 60% of total caloric intake for 24 weeks.

Group III (Exercise Group)
Received same as group II for 24 weeks and were exposed to exercise training for 30-45 minutes/day in the last 8 weeks (Jamine et al., 1993).

Group IV (Antioxidant Group)
Received high fat diet for 24 weeks and received quercetin at a dose of 15gm/kg intraperitoneal (IP) daily in the last 8 weeks (Khaki et al., 2009; Dias et al., 2005).

Group V (Exercise and Antioxidant Group)
Received high fat diet for 24 weeks and were exposed to exercise training for 30-45 minutes/day and received quercetin daily at a dose of 15g/kg (IP) in the last 8 weeks.

Composition of the Diet Used

Normal ( Balanced) Diet
In this type of diet, the fat represented 12% of the total caloric requirement while carbohydrates represented 69.4% of the total caloric requirement and protein represented 18.6% of the total caloric requirement (Timothy et al., 2005).

High Fat Diet
In this type of diet, fats represented 60% of the total caloric requirement, carbohydrates represented 21.4% of the total caloric requirement, and the protein represented 18.6% of the total caloric requirement (Timothy et al., 2005).

Chemicals Used

Quercetin Drug
Quercetin powder was obtained from Sigma Chemical Company (St. Louis, MO, USA). It was dissolved and diluted with 20% glycerol in 0.9% normal saline, mixed vigorously and stored in a dark bottle at 4°C. Quercetin solution was freshly prepared each week (Khaki et al., 2009).

Exercise Protocol
Our swimming protocol was established and was considered equivalent to moderate training (Craig and Foley 1984). Swimming exercise was started in a circular tank 80 cm in diameter and 90 cm in height were filled to 60 cm mark with 32-35°C water. The rats were initially acclimated to the water by 15 minute swim intervals. Training times then were slowly increased to 30-45 minutes over one week period (Jamine et al., 1993).

Assessment of Obesity
Body weights were recorded weekly during the experimental period, and before decapitation for all groups. Body lengths were measured (nose-to-anus or nose- anal length). The body weight and body length were used to confirm the obesity through the obesity parameters body mass index (body weight g/length cm2).

Procedure of the Experiments
At the end of the treatment period, the animals were anesthetized by sodium thiopental anesthesia (40 mg/kg, i.p.) after 12 hour fasting. The animals were fixed on an operating table and the blood samples were taken. The procedures were conducted as follows:

Blood Sample Collection
A craniocaudal incision of about 2 cm is made, parallel and slightly to the left of the sternum through the skin and pectoral muscles to expose the ribs. A blunt curved forceps is then binged between the 5th and 6th ribs through the intercostals muscles. The gap is widened so that the rapidly beating heart becomes visible, then the blood sample were taken from the right ventricle.

Biochemical Assessment
Blood samples were allowed to clot at room temperature and serum was separated by centrifugation at 3000 revolution per minute (rpm) for 15 min and stored at -20°C in dark containers for biochemical assessment:

Determination of Fasting Serum Glucose, Insulin, and Insulin Resistance
Fasting serum glucose was estimated by the glucose oxidase–peroxidase method (GOD–POD
EFFECT OF EXERCISE AND QUERCETIN ON OBESITY INDUCED METABOLIC AND...

Fasting serum insulin level was carried out using an enzyme linked immunosorbent assay kit (ELISA, Boehringer Mannheim Immunodiagnostics, and Mannheim, Germany).

**Calculation of the HOMA Index**

Insulin resistance was assessed by HOMA (homeostatic model assessment) using the following formula:

\[
HOMA - IR = \frac{Glucose}{18 \times \frac{Insulin}{22.5}}
\]

Where glucose is given in mg/dl and Insulin is given in µIU/ml (Matthews et al., 1985). A HOMA value that is > 2 is used to identify significant IR.

**Assessment of Renal Function**

Serum urea and creatinine was assessed using the Jaffe’ picric acid procedure with Sigma kit #555-A (Sigma-Aldrich Chemical Co.). Urine was collected for 24 hours after placing each rat in a metabolic cage and, to avoid urea degradation, urine samples were maintained frozen. Urinary albumin was measured by means of quantitative reaction using a sigma diagnostic kit.

**Determination of Serum Adiponectin**

The quantitative determination of serum adiponectin was performed using the mouse/rat adiponectin ELISA kit (B-Bridge International), according to the manufacturer's instructions (Kubota et al., 2002).

**Preparation of Renal Tissue Homogenate**

The previous incision was continued through the animal's anterior abdominal wall, the abdominal cavity was entered by cutting the muscles and peritoneum. The kidneys were exposed then freed from the surrounding tissue. Kidneys were quickly excised and portions of kidney tissues were homogenized in a saline solution (0.9%), centrifuged at 3000 rpm for 15 min, and the supernatant was kept at – 20°C and used for the determination of antioxidant parameters as MDA (Uchiyama and Mihara 1978), SOD (Das et al., 2000), reduced GSH (Moron et al., 1979), and pro-inflammatory cytokines (TNF-α) (Mohamed and Safwat 2013).

**Histopathological Examination**

Portion of kidney specimens fixed in formaline 10% and histopathological examination of them was done using Hematoxylin and Eosin (H&E) stain.

**Statistical Analysis**

The data are the mean ± SD. The data were processed and analyzed using the Statistical Package for the Social Sciences (SPSS) version 10.0 (SPSS, Inc., Chicago, Illinois, USA). A one-way ANOVA was performed followed by a Tukey’s Post Hoc test. The Pearson correlation statistical analysis was performed for detection of a probable significance between 2 parameters. The results were considered significant at the 95% Confidence Level (P<0.05).

**Results**

Body weights, body mass index, levels of serum glucose, insulin and HOMA measured in all groups are shown in Table 1. Regarding body weight and BMI, they were significantly increased in group II (obesity) (P<0.05) compared to group I (control), while they were significantly decreased in group III (exercise) & in group V (combined exercise and antioxidant) (P<0.05) compared to group II (obesity). However, no significant differences were found comparing group IV (antioxidant) and group II (obesity). Moreover, body weight and BMI were significantly reduced in group V (combined exercise & antioxidant) compared to group III (exercise) (P<0.05). Similarly body weight and BMI were significantly reduced in group III (exercise) compared to group IV (antioxidant). Serum glucose, insulin and HOMA insulin resistance were significantly increased in group II (obesity) (P<0.05) compared...
to group I (control), while these parameters were significantly decreased in group III (exercise) (P<0.05), group IV (antioxidant) (P<0.05) & group V (combined exercise & antioxidant) (P<0.05) compared to group II (obesity). There was significant reduction in serum glucose, serum insulin and HOMA insulin resistance in group V (combined exercise & antioxidant) (P<0.05) compared to group III (exercise) or group IV (antioxidant), although this reduction did not reach statistical significance comparing group III (exercise) and group IV (antioxidant). There was significant decrease in adiponectin in group II (obesity) (P<0.05) compared to group I (control), while it was significantly increased in group III (exercise) (P<0.05), group IV (antioxidant) (P<0.05) & group V (combined exercise & antioxidant) (P<0.05) compared to group II (obesity). Moreover, serum adiponectin was found to be significantly higher in group V (combined exercise & antioxidant) (P<0.05) compared to group III (exercise) and group IV (antioxidant), whereas there was significant increase in adiponectin in group III (exercise) than group IV (antioxidant).

Table 1: BMI, level of serum glucose, serum insulin, HOMA, and serum adiponectin in experimental groups (mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (Control)</th>
<th>Group II (Obesity)</th>
<th>Group III (Exercise)</th>
<th>Group IV (antioxidants)</th>
<th>Group V (antioxidants &amp; exercise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (gm)</td>
<td>200 ±7.65</td>
<td>355.57±34.26a</td>
<td>236.29±34.45b</td>
<td>323.57±24.30c</td>
<td>206.14±2.12bd</td>
</tr>
<tr>
<td>BMI (g/cm²)</td>
<td>024. ±0.54</td>
<td>0.87 ± 0.037b</td>
<td>0.64±0.035b</td>
<td>0.84± .029d</td>
<td>0.55±0.036bd</td>
</tr>
<tr>
<td>Serum Glucose (mg/dl)</td>
<td>97.71± 3.82</td>
<td>210.57±8.24a</td>
<td>125.86±4.49b</td>
<td>128.14±2.12b</td>
<td>99±3.70bcd</td>
</tr>
<tr>
<td>Serum insulin (μIU/ml)</td>
<td>4.66±0.97</td>
<td>6.25 ±1.52a</td>
<td>5.23±1.32b</td>
<td>5.34± 1.28b</td>
<td>4.01±0.70bcd</td>
</tr>
<tr>
<td>HOMA</td>
<td>1.03 ±0.24</td>
<td>3.16 ±0.71a</td>
<td>2.19±0.37b</td>
<td>2.15±0.42b</td>
<td>1.04±0.16bcd</td>
</tr>
<tr>
<td>Serum adiponectin (ng/ml)</td>
<td>24.3±0.97</td>
<td>12.5±0.28a</td>
<td>19.75±0.12b</td>
<td>17.29±0.47bd</td>
<td>23±0.63bcd</td>
</tr>
</tbody>
</table>

a: Significant difference (p<0.05) compared with control group.
b: Significant difference (p<0.05) compared with obesity group.
c: Significant difference (p<0.05) compared antioxidant group.
d: Significant difference (p<0.05) compared with exercise group.

Results of kidney functions including serum urea level, serum creatinine level, and urinary albumin excretion rate as well as serum level of adiponectin measured in all groups are shown in Table 2. There was significant increase in both serum urea, creatinine and urinary albumin levels in group II (obesity) (P<0.05) compared to group I (control), while they were significantly decreased in group III (exercise) (P<0.05), group IV (antioxidant) (P<0.05) & group V (combined exercise & antioxidant) (P<0.05) compared to group II (obesity). Moreover, serum urea, creatinine, and urinary albumin were significantly lower in group V (combined exercise & antioxidant) (P<0.05) compared to group III (exercise) and group IV (antioxidant), whereas no significant difference was found in urea and creatinine comparing group III (exercise) and group IV (antioxidant).

Table 1: Serum urea (BUN), serum creatinine levels and urinary albumin excretion rate measured in all experimental groups (mean ± SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (Control)</th>
<th>Group II (Obesity)</th>
<th>Group III (Exercise)</th>
<th>Group IV (antioxidants)</th>
<th>Group V (antioxidants &amp; exercise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum urea (mg/dl)</td>
<td>13.2±2.9</td>
<td>24.2±3.21a</td>
<td>18.9±3.11b</td>
<td>19.1±3.6b</td>
<td>13.85±2.54bcd</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.36± 0.02</td>
<td>3.61±0.24a</td>
<td>1.65±0.29b</td>
<td>1.86±0.26b</td>
<td>0.41±0.19bcd</td>
</tr>
<tr>
<td>Urinary albumin excretion rate (mg/24h)</td>
<td>0.42±0.08</td>
<td>2.3±0.26c</td>
<td>0.78±0.3b</td>
<td>1.03 ± 0.14bd</td>
<td>0.59±0.06bcd</td>
</tr>
</tbody>
</table>

a: Significant difference (p<0.05) compared with control group.
b: Significant difference (p<0.05) compared with obesity group.
c: Significant difference (p<0.05) compared antioxidant group.
d: Significant difference (p<0.05) compared with exercise group.
Table 3 shows renal MDA, antioxidant parameters including (SOD and reduced GSH) and renal pro-inflammatory cytokine TNF-α level. MDA content of the renal tissue was found to be significantly increased in group II (obesity) (P<0.05) compared to group I (control), while it was significantly decreased in group III (exercise) (p<0.05), group IV (antioxidant) (p<0.05) & group V (combined exercise& antioxidant) (p<0.05) compared to group II (obesity). The reduction of renal MDA was found to be significant (p<0.05) comparing group V (combined exercise& antioxidant) to group III (exercise) and group IV (antioxidant), while it was not significant comparing group III (exercise) and group IV (antioxidant). The SOD and reduced GSH contents of the kidney in Group II (obesity) were significantly decreased compared to group I (control) (P<0.05), while they were significantly increased in group III (exercise) (p<0.05), group IV (antioxidant) (p<0.05) & group V (combined exercise& antioxidant) (p<0.05) compared to group II (obesity). Moreover, there was significant increase in renal SOD and reduced GSH in group V (combined exercise& antioxidant) (p<0.05) compared to group III (exercise) and group IV (antioxidant), but no significant difference was found comparing group III (exercise) and group IV (antioxidant).

The level of renal TNF-α was significantly increased in group II (obesity) (P<0.05) compared to group I (control), but it was significantly decreased in group III (exercise) (p<0.05), group IV (antioxidant) (p<0.05) & group V (combined exercise& antioxidant) (p<0.05) compared to group II (obesity). Also, there was significant reduction in renal TNF-α in group V (combined exercise & antioxidant) (P<0.05) compared to group III (exercise) or group IV (antioxidant), whereas this reduction did not reach statistical significance in comparing group III (exercise) and group IV (antioxidant).

**Table 3:** MDA, SOD, GSH, and TNF-α level measured in all experimental groups(mean ±SD).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group I (Control)</th>
<th>Group II (Obesity)</th>
<th>Group III (Exercise)</th>
<th>Group IV (antioxidants)</th>
<th>Group V (antioxidants &amp;exercise)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (nmol/g tissue)</td>
<td>6.75±1.65</td>
<td>12.57±4.26</td>
<td>8.29 ±3.45</td>
<td>8.31 ± 3.26</td>
<td>6.97±2.12bcd</td>
</tr>
<tr>
<td>SOD (U/mg protein)</td>
<td>7.05 ±0.55</td>
<td>3.87 ± 0.03a</td>
<td>5.59±0.04</td>
<td>5.67 ±0.03b</td>
<td>6.91±0.03bcd</td>
</tr>
<tr>
<td>Reduced GSH (U/mg protein)</td>
<td>5.71±0.32</td>
<td>2.37±0.04a</td>
<td>4.06±0.49b</td>
<td>4.14±0.12b</td>
<td>5.04±0.7bcd</td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>134.6±8.97</td>
<td>194.5 ±6.52a</td>
<td>167.8 ±2.32b</td>
<td>163.4 ± 1.48b</td>
<td>138.1±4.07bcd</td>
</tr>
</tbody>
</table>

a: Significant difference (p<0.05) compared with control group.
b: Significant difference (p<0.05) compared with obesity group.
c: Significant difference (p<0.05) compared antioxidant group.
d: Significant difference (p<0.05) compared with exercise group.

**Histopathological Finding**

*Group I:* Showed normal appearance of the glomeruli & tubules (Fig. 1).
Group II: Showed new capillary proliferation, lymphocytic infiltration, interstitial tissue necrosis and tubular hyalinosis. (Fig. 2).

Group III: Showed mild improvement of interstitial tissue necrosis and tubular hyalinosis but there is mesengeal proliferation (Fig. 3).

Group IV: Showed mild improvement of capillary proliferation and tubular hyalinosis but there is interstitial hemorrhage (Fig. 4).

Group V: Showed normal appearance of the glomeruli & interstitial tissue (Fig. 5).

Discussion

High-fat diet-induced obesity is accompanied by increased renal tissue oxidative stress and inflammatory reaction with decrease in adiponectin level. Therefore, it might be assumed that moderate exercise and antioxidant supplementations could play an important role on renal morphological and functional alterations induced by obesity.

In the current study, obesity induced by high fat diet for 24 weeks (Group II) resulted in a significant increase in body weight and BMI associated with insulin resistance manifested by a significant increase in plasma glucose level, plasma insulin level and HOMA insulin resistance with a significant decrease in serum adiponectin level when compared with control rats (group I). Our findings are in agreement with Timothy et al., (2005) and Barnea et al., (2006) as they reported that high fat diets produce insulin resistance in experimental animals assessed by weight gain, increased plasma glucose level, plasma insulin level and elevated HOMA. Obesity-induced insulin resistance could be explained by different mechanisms such as endocrinal mechanisms where the insulin sensitizing adipokines (adiponectin) decrease (Diez and Iglesias 2003) while adipokines increasing insulin resistance (Interleukin-6, Tumor necrosis factor, Plasminogen activator inhibitor-1, and resistin) increase (Qi et al., 2006). TNF-α may be the mechanism by which insulin resistance is developed in obesity as it increases the release of free fatty acids (FFA) in adipocytes, it blocks the synthesis of adiponectin, and it interferes with the activity of tyrosine-residue phosphorylation activity in the first substrate of the insulin receptor, which is necessary for progression of the intracellular signal of the hormone (Lastra et al., 2006). This explanation could be confirmed by our results as TNF-α levels in high fat diet groups were significantly increased with the significant decrease in adiponectin.

Exercise training for 45 minutes/day for 8 weeks (group III) resulted in a significant decrease in body weight and BMI associated with an improvement in the insulin resistance state as there was significant decrease in plasma glucose level, plasma insulin level, and HOMA while there was significant increase in adiponectin on comparing them with the obesity group (group II). This improvement in insulin resistance under the effect of exercise was reported by Huang et al., (2006); Lee et al., (2005); and Sun et al., (2009) who reported that exercise training improves resistance to insulin induced by high-fat diets. Several Mechanisms have been proposed to account for this exercise-related improvement in the insulin resistant state. Saengsirisuwan et al., (2002) have proposed many preceptor events as increased muscle glucose delivery because of increased muscle capillary density and changes in muscle composition favoring increased glucose disposal as well as postreceptor adaptations as enhanced glucose transport via increased concentration of GLUT-4 in skeletal muscle and greater activity of the enzymes hexokinase II and glycogen synthase (Graham et
Furthermore, Kizer et al., (2008) showed that adiponectin improves insulin sensitivity and decreases the adverse effects of inflammatory mediators. Therefore, the insulin-sensitizing effects of exercise are associated with increased circulating adiponectin. In the contrary of our results, other researchers have shown that exercise alone is not effective for weight loss (Chow et al., 2007; Georgieva and Boyadjiev 2004).

By studying the effect of quercetin supplementation at a dose of 15 gm/kg for 8 weeks (group IV), it resulted in an improvement in insulin resistant states as there was a significant decrease in serum glucose level, serum insulin levels, and HOMA with a significant increase in adiponectin, while there was no significant decrease regarding body weight or BMI when compared to the obesity group (group II). These results coincide with Arias et al., (2014) and Leonor et al., (2008) who reported that quercetin caused no significant reduction in body weight or adipose tissue sizes. However, serum glucose, serum insulin, and consequently HOMA-IR, were significantly reduced with significant increase in adiponectin by quercetin. Positive effect of quercetin on glycemic control could be explained by several mechanisms including; the anti-oxidative protective action on the pancreatic islets (Coskun et al., 2005; Babujanarthanam et al., 2010; Jeong et al., 2012), the increase in adiponectin circulating concentration (Wein et al., 2010), the inhibition of small intestine glucosidase activity (Kim et al., 2011), the reduction in the intestinal glucose absorption mediated by GLUT2 (Kwon et al., 2007), the increase in glucokinase activity (Vessal et al., 2003) and the increase in GLUT4 transporters in skeletal muscle (Jung et al., 2011; Shen et al., 2012; Anhe` et al., 2012).

Concerning kidney functions, obese rats (group II) showed a significant increase in the concentration of serum urea, creatinine and urinary albuminuria compared with the control group (group I) and confirmed histopathologically by new capillary proliferation, lymphocytic infiltration, interstitial tissue necrosis and tubular hyalinosis which is in agreement with the results of Muhammed et al., (2008), Cindik et al., (2005) and Amin et al., (2011). As obesity is associated with an insulin resistant state, the pathophysiology involves glucose that binds irreversibly to proteins in the kidney circulation to form advanced glycosylation end products (AGEs) that can form complexes that contribute to renal damage by stimulation of fibrotic growth factors (Rao and Nammi 2006). High fat diets (HFD) induce alteration of renal lipid metabolism by an imbalance between lipogenesis and lipolysis in the kidney as well as systemic metabolic abnormalities and subsequent renal lipid accumulation leading to renal injury (Kume et al., 2007). In addition HFD resulted in hyperinsulinemia, activation of the renin-angiotensin system, glomerular hyperfiltration and structural changes in the kidney that may be the precursors of more severe glomerular injury associated with prolonged obesity (Tokuyama et al., 2008). Subjects with severe obesity develop proteinuria with pathologic findings of podocyte hypertrophy, mesangial expansion, glomerular enlargement, and focal segmental glomerular sclerosis (Serra et al., 2008).

Serum urea, creatinine, and albuminuria were significantly decreased in exercise group (group III) comparing it with the group receiving high fat diet (group II) with mild improvement in renal histopathology. Our findings were in agreement with (Lin et al., 2011) who reported that regular exercise leads to the reduction of blood urea nitrogen levels and serum creatinine and consequently improves the renal disorders. Also, Park et al., (2013) suggested that regular exercise protects against obese and oxidative stress-related renal injury and restored all renal changes in HFD rats. Toyama et al., (2010) suggested that the potential clinical benefits of regular exercise can correlate with improving renal function through modifying lipid metabolism, particularly HDL elevation. In addition, moderate regular exercise reduced the burden of AGEs in obesity-associated nephropathy rats (Boor et al., 2009). Yuji et al., (2012) reported that low-intensity exercise improved albuminuria through maintaining podocyte numbers, with parallel improvements in oxidative damage and chronic inflammation.

To the best of our knowledge, this is the first report that analyzes the effect of quercetin on the renal function of high fat diet induced obesity in
rats. This study revealed significant decrease in serum urea, creatinine and albuminuria in antioxidant group (group IV) when compared to obesity group (group II). Our findings were in agreement with Ginpreet and Meena (2012) & Bin-Lai et al., (2012) who stated that quercetin improved renal morphology and functions, including serum urea and creatinine in rats with diabetic nephropathy. This can be explained by the antioxidant potential of quercetin (Meyers et al., 2008; Annapurna et al., 2009; Yao et al., 2010). The antioxidant activity of quercetin relies on its ability to act as scavenger molecule, as well as to inhibit ROS-generating enzymes and to enhance expression of antioxidant enzymes (Savini et al., 2013).

As regard oxidative stress parameters, there was significant increase in MDA as well as a significant reduction of antioxidant enzymes (SOD and reduced GSH) suggesting the development of renal oxidative stress in HFD-fed rats (group II) compared to control group (group I). These results are in line with the findings reported by Darouich et al., (2011) who reported that Oxidative stress has been commonly identified in obesity-related renal diseases and may be the mechanism underlying the initiation or progression of renal injury in obesity. Obesity-induced oxidative stress could be explained by different mechanisms including, the presence of excessive adipose tissue itself, as adipocytes has been identified as a source of proinflammatory cytokines, including TNF-α, IL-1, and IL-6. These cytokines are potent stimulators for the production of ROS. Another explanation was showed by Khan et al., (2006) who reported that obesity increases the mechanical load and myocardial metabolism; therefore, oxygen consumption is increased. One negative consequence of increased oxygen consumption is the production of ROS. Khan et al., (2006) also introduced excessive fat accumulation as a cause of cellular damage due to the pressure effect from fat cells. Cellular damage in turn leads to high production of cytokines which generates ROS in the tissues.

Regular exercise training (group III) resulted in a significant decrease in renal MDA level associated with an improvement in the antioxidant state manifested by significant increase in SOD and reduced GSH levels on comparing them with the group receiving HFD (group II). These results highly suggest that exercise has an important role in decreasing oxidative damage and increasing resistance to oxidative stress. This improvement under the effect of exercise was reported by Park et al., (2013) and Cooper et al., (2002). In fact, regular exercise causes adaptations in the antioxidant capacity, protecting cells against the harmful effects of oxidative stress, thus preventing cellular damage (Golbidi et al., 2012). It also exerts an anti-inflammatory action increasing anti-inflammatory cytokine IL-1 and IL-10 levels and reducing generation of the pro-inflammatory cytokine TNF-α (Hopps et al., 2011). Therefore, regular physical activity appears to act as a natural antioxidant and anti-inflammatory strategy for preventing obesity-associated complications. It improves glucose-insulin homeostasis and antioxidant defenses (Savini et al., 2013).

There was significant reduction in the oxidative stress markers in rats treated with Quercetin (group IV) compared to rats receiving HFD (group II). This was proven by the significant reduction in renal MDA levels with concomitant elevations in the renal antioxidant enzymes SOD and reduced GSH activities. This finding is completely in agreement with those of (Bashir et al., 2014) who demonstrated antioxidant activity of quercetin in diabetic rats. Suggested mechanisms that are related to quercetin’s potent antioxidant potential directly scavenging ROS and other free radicals have been proposed by Mira et al., (2002) and Tong-un et al., (2013).

Consumption of high fat diet (group II) was associated with significant increase in TNF-α level in the kidney. Our findings were in agreement with Fernández-Sánchez et al., (2011) who stated that obesity is considered a state of chronic inflammation because adipocytes has been identified as a source of proinflammatory cytokines, including TNF-α. Another possible explanation is that triglycerides and/or free fatty acids may be inducers of TNF-α expression because feeding rats a high-fat diet resulted in a significant increase in TNF-α, mRNA, and protein in fat pads (Speretta et al., 2012). Also, Speretta et al., (2012) showed that the high-fat diet induced a higher (six-fold) TNF-α
level in visceral adipose tissue.

TNF-α in the kidney was significantly decreased among exercise group (group III) comparing it to obesity group (group II). These findings mean that increased physical activity for 8 weeks is sufficient for decreasing TNF-α level. Our results are in agreement with several experimental studies such as Speretta et al., (2012) and Starkie et al., (2003). Furthermore, exercise also suppresses secretion of TNF-α by pathways independent of IL-6, as shown by the results obtained with knockout mice for IL-6 submitted to exercise. Another explanation was made by Gleeson et al., (2011) who stated that the anti-inflammatory effects of regular exercise may be mediated via both a reduction in visceral fat mass (with a subsequent decreased release of adipokines) and the induction of an anti-inflammatory environment with each bout of exercise.

Quercetin administrated rats (group IV) showed a significant decrease in TNF-α levels compared to obese rats (group II). The findings of Rivera et al., (2008) supported our results about the anti-inflammatory effect of quercetin. Quercetin ameliorates the inflammatory response induced by high-fat diets (Stewart et al., 2008). Quercetin reduced visceral adipose tissue TNF-α and nitric oxide production in obese Zucker rats (Rivera et al., 2008).

By studying the effect of combined exercise training and quercetin supplementation (group V), there was a significant improvement in all parameters by comparing it with HFD-fed group (group II), exercise group (group III), and antioxidant group (group IV). We thought that the additive effect of the two interventions (exercise training combined with quercetin supplementation) is associated with greater improvements than either intervention individually. These results confirmed histopathologically as combination of exercise training and quercetin showed normal appearance of the glomeruli & interstitial tissue. Furthermore, quercetin supplementation may improve exercise performance as shown by Poya et al., (2013). Moreover, exercise is superior to quercetin in decreasing body weight and BMI.

We concluded that exercise training and antioxidant treatment improved HFD induced insulin resistance, oxidative stress; inflammatory reaction in renal tissue with increase in adiponectin level. And the maximum improvement was among combined exercise& antioxidant group.

References


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