The Water Extract of *Mytilus edulis* Suppresses Oxidative Stress and Improves Male Reproduction Dysfunction in High-Fat Diet-induced Obese Rats

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**Abstract:** Obesity is a complex metabolic disease, which induces a variety of diseases, such as cardiovascular disease, type 2 diabetes, hypertension, and cancer. Various chemical agents can be used for treat obesity, but adverse some side effects, such cardiovascular disease and stroke. Natural products reported possessing anti-obesity activity. *Mytilus edulis* (ME) has been associated with some functional properties such as antioxidant and anti-inflammation. This study aims to determine the preventive effects of *Mytilus edulis* water extract (MWE) on male reproduction dysfunction in high-fat diet (HFD)-induced obesity rats. The Sprague-Dawley (SD) rats used for obesity model and fed by HFD for 13 weeks. The rats were treated with three different doses of MWE (MWE1, 150 mg/kg body weight; MWE2, 300 mg/kg; MWE5, 750 mg/kg). The results showed that the MWE can reduce body weight, total cholesterol (TC), and triglyceride (TG). In reproduction function, MWE showed that the percentage of sperm motility and progressive motility significantly increase compared to no treated-HFD group. MWE was also reduced oxidative stress and increase the enzymatic antioxidant activity such as superoxide dismutase (SOD) and glutathione peroxidase (GPx). In the conclusion suggested that MWE had beneficial effects on the male reproduction dysfunction in HFD-induced obese rats.

**Keywords:** Male reproduction; High-fat diet; *Mytilus edulis*; Obesity.

1. Introduction

Obesity is a medical condition with excessive fat accumulation, which the results increasing the body weight with body mass index (BMI) ≥ 30 kg/m². Obesity is a metabolic disorder and characterized by excess triglycerides (TG) in adipose tissue [1,2], which may induce the inflammatory mediator, such as leptin, resistin, monocyte chemoattractant protein-1 (MCP-1), and tumor necrosis factor-α (TNF-α) [3]. The reducing of adiponectin level also associated with obesity [4]. Obesity have induced numerous of chronic disease including diabetes mellitus, hypertension, osteoarthritis, cardiovascular disease, and some type of cancers [2,5]. Insulin resistant and type 2 diabetes also considered causing by obesity [6]. Moreover, hyperinsulinemia and hyperglycemia condition also common occurrence in obese condition. This condition has been shown the inhibitory effect on sperm quantity and quality; and as the result it could be attributing to the reduced the fertility [7,8]. Obesity also have been associate with low testosterone level. Luteinizing hormone (LH) and local paracrine control regulates the expression level of testosterone in testis [9].

Anti-obesity agents can able to alleviate the energy absorption and decreased the fat mass by increasing energy expenditure [10]. Various chemical agents have been used for anti-obesity such as sibutramine and orlistat, but they have some adverse effects such as constipation, cardiovascular disease, thirst, headache, steatorrhea, insomnia, and strokes [11-13]. According this condition, there is an urgent for alternative therapeutically agent for anti-obesity drugs. With the result that, some natural products have been increasing interest for treated obesity. Marine organisms have been found as an alternative for obesity treatment with their bioactive compounds [1,14].
The blue mussel (*Mytilus edulis*) is belongs to family Mytilidae also known as black mussel and widely distributed from European to Asian water [15,16]. The major compound of this mussel is protein and has been reported that bioactive peptide from *M. edulis* showed the antioxidant activities against the hydroxyl radical-induced DNA damage and inhibited the NO production [15]. This mussel also reported has high content of eicosapentaeanoic acid (EPA) and docosahexaenoic acid (DHA). In addition, *M. edulis* also high nutrient content such as carotenoids, riboflavin, selenium, and zinc [17,18]. Another study showed that fatty acid from *M. edulis* possess anti-inflammatory activity in arthritis rats without adverse side effects [18]. The most recent study, reported that supplementation of *M. edulis* can reduced the pain and fatigue in patient with rheumatoid arthritis (RA) [19]. However, effects of *Mytilus edulis* water extract on male reduction dysfunction in obese rats haven’t been reported. Based on this condition, this study aims to investigate the effects of *Mytilus edulis* water extract (MWE) on male reproduction dysfunction in high-fat diet (HFD)-induced obese rats.

2. Results

2.1. Effects of MWE on the body and organs weight

During experiment, the body weight of the rats fed with high-fat diet (HFD) higher than control group (Figure 1). After treated with MWE (especially from 10th week of age of rat), the rats have lower body weight compared than untreated HFD-induced obese rats. Treatment with MWE can reduce the body weight of rats.

![Figure 1. Effects of MWE on body weight in HFD-induced rat during experiment. Data are showed as the mean ± S.D. (n = 6). * p < 0.05; ** p < 0.01. versus HFD group.](image)

Oral administration of MWE had no any significant different effects on the spleen, heart, and kidney, but in liver shows the significantly (*p*<0.05) decreased and increased in the testis weight compared than untreated HFD-induced obese rats (Table 1). In high concentration administration of MWE can reduced the epididymal and retroperitoneal fat and shows significant (*p*<0.05) different compared with untreated HFD group.

<p>| Table 1. Effect of MWE on the relative weight of organs and white adipose tissue in HFD-induced obese rats after 6 weeks. |</p>
<table>
<thead>
<tr>
<th>Organ weight (g)</th>
<th>Control</th>
<th>HFD</th>
<th>MWE1</th>
<th>MWE2</th>
<th>MWE5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spleen</td>
<td>0.12 ± 0.01 *</td>
<td>0.13 ± 0.01 *</td>
<td>0.12 ± 0.02 *</td>
<td>0.13 ± 0.01 *</td>
<td>0.13 ± 0.01 *</td>
</tr>
<tr>
<td>Heart</td>
<td>0.29 ± 0.01 *</td>
<td>0.28 ± 0.01 *</td>
<td>0.29 ± 0.02 *</td>
<td>0.28 ± 0.02 *</td>
<td>0.27 ± 0.02 *</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.69 ± 0.03 *</td>
<td>0.69 ± 0.01 *</td>
<td>0.69 ± 0.02 *</td>
<td>0.67 ± 0.05 *</td>
<td>0.69 ± 0.01 *</td>
</tr>
</tbody>
</table>
Liver & 2.55 ± 0.06 \textsuperscript{a} & 2.60 ± 0.12 \textsuperscript{b} & 2.66 ± 0.11 & 2.53 ± 0.07 \textsuperscript{a} & 2.52 ± 0.06 \textsuperscript{a} \\
Testis & 0.64 ± 0.03 \textsuperscript{a} & 0.57 ± 0.01 \textsuperscript{b} & 0.59 ± 0.02 \textsuperscript{b} & 0.59 ± 0.01 \textsuperscript{b} & 0.65 ± 0.03 \textsuperscript{a} \\

The relative weight of adipose tissue (% of body weight)

Epididymal fat & 1.40 ± 0.07 \textsuperscript{c} & 2.43 ± 0.10 \textsuperscript{a} & 2.32 ± 0.4 \textsuperscript{ab} & 2.12 ± 0.08 \textsuperscript{ab} & 2.04 ± 0.24 \textsuperscript{b} \\
Retroperitoneal & 2.24 ± 0.25 \textsuperscript{a} & 3.43 ± 0.37 \textsuperscript{b} & 3.21 ± 0.24 \textsuperscript{b} & 3.00 ± 0.46 \textsuperscript{b} & 2.41 ± 0.47 \textsuperscript{a} \\

Data are showed as the mean ± S.D. (\(n = 6\)). The values with different superscript letters (a-c) represent significantly different (\(p<0.05\)) via one-way ANOVA, analyzed by Duncan’s multiple range test.

2.2. Effect of MWE on plasma lipid

Plasma lipid properties was measured after sacrificed the rat includes plasma triglycerides (TG), total cholesterol (TC), HDL-Cholesterol, and LDL-Cholesterol. After 6-weeks treatment with MWE, the level of TC, TG, and LDL-Cholesterol was significantly \((p<0.05)\) decreased, whereas HDL-Cholesterol was increased compared than untreated HFD-induced obese rats (Table 2).

<table>
<thead>
<tr>
<th>Parameters (mg/dl)</th>
<th>Control</th>
<th>HFD</th>
<th>MWE1</th>
<th>MWE2</th>
<th>MWE5</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>47.55 ± 9.55 \textsuperscript{b}</td>
<td>64.35 ± 3.81 \textsuperscript{a}</td>
<td>61.84 ± 10.61 \textsuperscript{a}</td>
<td>52.86 ± 10.35 \textsuperscript{a}</td>
<td>53.64 ± 4.52 \textsuperscript{b}</td>
</tr>
<tr>
<td>TC</td>
<td>92.33 ± 5.39 \textsuperscript{b}</td>
<td>106.52 ± 9.11 \textsuperscript{a}</td>
<td>97.09 ± 5.86 \textsuperscript{b}</td>
<td>87.18 ± 9.08 \textsuperscript{b}</td>
<td>88.77 ± 4.84 \textsuperscript{b}</td>
</tr>
<tr>
<td>HDL-C</td>
<td>2.15 ± 0.29 \textsuperscript{b}</td>
<td>1.70 ± 0.08 \textsuperscript{a}</td>
<td>1.49 ± 0.10 \textsuperscript{a}</td>
<td>1.69 ± 0.11 \textsuperscript{a}</td>
<td>2.10 ± 0.16 \textsuperscript{b}</td>
</tr>
<tr>
<td>LDL-C</td>
<td>0.92 ± 0.09 \textsuperscript{b}</td>
<td>2.14 ± 0.18 \textsuperscript{a}</td>
<td>1.09 ± 0.12 \textsuperscript{b}</td>
<td>1.01 ± 0.09 \textsuperscript{b}</td>
<td>1.03 ± 0.14 \textsuperscript{b}</td>
</tr>
</tbody>
</table>

HDL-C, High-density lipoprotein-cholesterol; LDL-C, Low-density lipoprotein-cholesterol. Data show as the means ± S.D. (\(n = 6\)). The values with different superscript letters (a-c) represent significantly different (\(p<0.05\)) via one-way ANOVA, analyzed by Duncan’s multiple range test.

2.3. Effect of MWE on insulin and inflammatory cytokine expression

In rats fed with HFD shows the significantly \((p<0.05)\) increased of insulin and leptin level, whereas decreased the adiponectin level (Figure 2). However, after treated with MWE, the level of insulin and leptin was significantly \((p<0.05)\) reduce and the value become same with control. In addition, the level of adiponectin increased significantly compared than untreated HFD group.

![Figure 2](https://example.com/figure2.png)

Figure 2. Effects of MWE on insulin, leptin, and adiponectin expression in HFD-induced rat after treatment for 6 weeks: (a) Insulin; (b) Leptin; (c) Adiponectin. Data are showed as the mean ± S.D. (\(n = 6\)).

2.4. Effects of MWE on male reproduction properties

In this study, we measured some the male reproduction properties includes the enzymatic antioxidant activity (superoxide dismutase, SOD; glutathione peroxidase, GPx), reproduction hormone (follicle-stimulating hormone, FSH; luteinizing hormone, LH; testosterone, T), and the sperm parameters (sperm count; motility; abnormality).

2.4.1 Enzymatic antioxidant activity
The level of superoxide dismutase (SOD) and glutathione peroxidase (GPx) was significantly \( p<0.05 \) decreased in untreated HFD group compared than control groups (Figure 3). Oral administration of MWE increased the level of GPx antioxidant and the values become no any significant different with control group. It also increased the SOD level, but no any significant different with untreated HFD group.

![Figure 3](image_url)

**Figure 3.** Effects of MWE on enzymatic antioxidant activity in HFD-induced rat after treatment for 6 weeks: (a) Superoxide dismutase; (b) Glutathione peroxidase. Data are showed as the mean ± S.D. \( (n = 6) \).

### 2.4.2. Reproductive hormones

The results of reproduction hormones analysis showed that supplementation with MWE can significantly \( p<0.05 \) increase the testosterone level in HFD-induced obese rats (Figure 4). In addition, the LH level was higher in treated HFD groups compared than untreated HFD group, but no any significant different. And MWE had no effected on FSH level in rat testis.

![Figure 4](image_url)

**Figure 4.** Effects of MWE on reproductive hormone in HFD-induced rat after treatment for 6 weeks: (a) FSH; (b) LH; (c) Testosterone. Data are showed as the mean ± S.D. \( (n = 6) \).

### 2.4.3. Sperm properties

After treated with MWE, the sperm count and motility were significantly \( p<0.05 \) increased compared than untreated HFD group (Figure 5). Whereas, the percentage of abnormality was significantly \( p<0.05 \) reduced after treated with MWE for 6 weeks.

![Figure 5](image_url)

**Figure 5.** Effects of MWE on sperm properties in HFD-induced rat after treatment for 6 weeks: (a) Sperm number; (b) Motility; (c) Abnormality. Data are showed as the mean ± S.D. \( (n = 6) \).
Figure 5. Effects of MWE on sperm properties in HFD-induced rat after treatment for 6 weeks: (a) Sperm number; (b) Motility; (c) Abnormality. Data are showed as the mean ± S.D. (n = 6).

2.5. Testis histopathology

Under the hematoxylin and eosin (H&E) staining observation, the testicular morphology shows the different between each group (Figure 6). In rats induced with HFD, shows the seminiferous tubule structure appeared shrunken and separated from each other. After treated with MWE for 6 weeks, the tubular diameter and space each was reduced compared than untreated HFD group.

Figure 6. Effects of MWE on testicular histopathology in HFD-induced rat after treatment for 6 weeks.

3. Discussion

Obesity is a major health problem in the world. Obesity is associated with developing metabolic disorder such as hypertension, diabetes, cancer, and cardiovascular diseases [20-22]. The obesity development is characterized by increasing the body weight with body mass index (BMI ≥ 30 kg/m²) with excessive the fat accumulation, excess the triglycerides (TC) level in adipose tissue [2]. In rats fed with high-fat diet (HFD) showed the higher body weight compared than control group (Figure 1). After treatment with MWE extract for 6 weeks, can reduce the body weight with the values lower than untreated HFD group. Following the increasing of body weight, adipose tissues accumulation includes the epididymal and retroperitoneal fat also increase in HFD group and treated with MWE reduce the tissue weight (Table 1). Previous study reported that blue mussel (Mytilus edulis) water extract showed the antiadipogenic activity and inhibited the lipid accumulation by suppressed expression of mRNA level of adipogenic genes such as peroxisome proliferator-activated receptors (PPAR)γ, CCAAT/enhancer-binding protein (C/EBP)-β, and C/EBP-α [16]. This study found that MWE treatment results in a marked reduce the body weight and fat accumulation in HFD-induced obese rat, this condition suggesting anti-obesity effect of MWE.

In plasma lipid analysis showed the increasing of triglycerides (TG), total cholesterol (TC), and low-density lipoprotein (LDL)-cholesterol as well as decreasing the high-density lipoprotein (HDL)-cholesterol in HFD-induced obese rats (Table 2). The increasing of TG, TC, and LDL-cholesterol is associated with obesity. Cardiovascular disease also associated by increased the triglycerides and total cholesterol [21,23]. Oral administration of MWE regulated the acclamation of cholesterol and triglycerides in HFD-induced obese rats. In addition, MWE administration increase the level of HDL-cholesterol. Previous study reported that exercise and diet induced the weight loss and followed by raising the level of HDL-cholesterol [24]. Obesity also associated with increasing the level of insulin
and leptin [25-27]. In rat treated with MWE reduced the insulin and leptin level (Figure 2a and 2b). The weight loss associated with decreasing of leptin level [26]. Beside accompanied by insulin resistant, obesity also accompanied by increases of inflammatory marker expression and oxidative stress [27]. HFD group decreased the enzymatic antioxidant activity such us superoxide dismutase (SOD) and glutathione peroxidase (GPx) and treated with MWE raising these antioxidant activity (Figure 3). In addition, MWE treatment also increases the adiponectin level (Figure 2c). Previous studies reported that adiponectin can acts at inhibition of the pro-inflammatory cytokines or induction the anti-inflammatory and the low adiponectin level associated with obesity [4,28].

In testicular analysis, HFD-induced rats showed the decrease level of gonadal hormone, especially testosterone level. The low level of total and free testosterone and low sexual hormone-binding globulin (SHBG) are associated with metabolic syndrome, type 2 diabetes, increased fat mass, and obesity [9,29,30]. In more severe obesity, its caused by suppression of the hypothalamic-pituitary-testicular (HPT) axis [31]. Oral administration of MWE showed the increased level of testosterone in HFD-induced obese rats (Figure 4). Under microscopic observation, we counted and found the sperm number and motility of sperm decrease in HFD-induced obese rats, whereas increase the sperm abnormality (Figure 5). The level of enzymatic antioxidant such SOD and GPx were decrease in HFD-induced obese rats (Figure 3). Increasing of oxidative stress in fat accumulation (obesity) is an important pathogenic system of obesity-associated metabolic syndrome [32]. Oxidative stress has been recognized as one of the mediator of male infertility due to sperm dysfunction [33]. Enhancement level of reactive oxygen species (ROS) acts at induction of abnormal spermatozoa and can be main cause of sub-fertility and even infertility. In addition, increasing production of ROS correlated with decreased level of enzymatic antioxidant [32,34]. The level of enzymatic antioxidants increases after treatment with MWE for 6 weeks and showed the positive correlation with increasing the sperm count and motility and it also reduced the sperm abnormality (Figure 3 and 5). Under hematoxylin and eosin (H&E) staining, in HFD-induced obese rat showed decrease the diameter seminiferous tubule and increase the space each other (Figure 6). After treated with MWE, the space was reduced and increased the seminiferous tubule diameter. Overall, treated with MWE ameliorated the male reproduction dysfunction marker in HFD-induced obese rats.

4. Materials and Methods

4.1. Blue mussel (Mytilus edulis) preparation and extraction

The blue mussel (Mytilus edulis) harvested from Matsu Island, Taiwan. The mussel extracted by boiling water extraction followed the previous methods [16] with a modification. Briefly, the whole of blue mussel extracted with boiling water (1:2, w/v) for 1 h and separated the filtrate and waste. The extraction was performance two times with same condition. The filtrates were dried use freeze-dryer machine and the results is Mytilus edulis water extract (MWE).

4.2. Animal experiment

Five-week-old male Sprague-Dawley (SD) rats were purchased from BioLASCO Taiwan, Co., Ltd and the average weight approximately 150 g. the rats were acclimatized for 1 week in experimental facility and maintained at 22°C with 55% of relative humidity. The room was exposed to alternating 12 h periods of light and dark. The protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan Ocean University (IACUC Approval No. 107015). After acclimatization phase, the rats randomly divided into 5 groups with 6 rats per group. The control group was fed by standard chow-fed diet (LabDiet® 5001 Rodent Diet; composed of 13.38% kcal from fat, 57.95% kcal from carbohydrates, and 28.67% kcal from protein) and other groups (Obese groups) were fed with high-fat diet (HFD, composed of 45.00% kcal from fat, 36.04% kcal from carbohydrates, and 18.97% kcal from protein). The rats were fed for 7 weeks before treated or untreated with Mytilus edulis water extract (MWE). After 7 weeks allowed free access water and HFD, a group from obese group was orally by gastrointestinal tract only by water (HFD group) and other obese groups were orally by three different doses of MWE extract; 150
mg/kg body weight, MWE1 group; 300 mg/kg, MWE2 group, and 750 mg/kg, MWE5 group. The treatment was performed for 6 weeks. The food intake and body weight were measure every week. The rats were sacrificed at 19 weeks of the age by exposure to CO\textsuperscript{2} in an empty chamber until euthanasia. All the rats fasted for 12 h before sacrifice. The whole blood sample was collected and the organs such as testis, epididymis, fat, heart, liver, kidney, and spleen weighted at the day of sacrifice.

4.3. Sample collection

The blood sample centrifuged at 3000x g for 10 min and collected the blood plasma. Representative of testis from each group was fixed by 10% neutral buffered formalin solution for histopathology analysis. The other testis and blood plasma were stored at -20°C for future analysis.

4.4. Blood plasma analysis

Triglyceride (TG) levels in plasma were measured using commercial kits (TR210, Randox Laboratories, Crumlin, Antrim, UK). Total cholesterol (TC) levels in plasma were measured using commercial kits (CH7945, Randox Laboratories, Crumlin, Antrim, UK). Levels of high-density lipoprotein (HDL)-cholesterol and low-density lipoprotein (LDL)-cholesterol were measured using HDL-cholesterol and LDL-cholesterol assay kits (BXC0422A & BXC0432A, Fortress Diagnostics Limited, Antrim, Northern Ireland, UK). Level of insulin was measured using commercial kits (ERINS, Thermo Fisher Scientific, San Jose, CA, USA). Leptin level was measured using commercial kits (ab100773, Abcam, Cambridge, Massachusetts, USA). Levels of adiponectin was measured using commercial kits (SEA605Ra, USCN Life Science, Wuhan, China). Testosterone levels in plasma were measured using commercial kits (ab108666, Abcam, Cambridge, Massachusetts, USA). Follicle-stimulating hormone level was measured using commercial kits (MBS017508, Mybiosource, San Diego, California, USA). Luteinizing hormone level was measured using commercial kits (MBS700807, Mybiosource, San Diego, California, USA). Superoxide dismutase level was measured using commercial kits (SD125, Randox Laboratories, Crumlin, Antrim, UK). Glutathione peroxidase level was measured using commercial kits (RS505, Randox Laboratories, Crumlin, Antrim, UK).

4.5. Sperm analysis

4.5.1. Sperm preparation

We used swim-up method to preparation sperm as described by previous method [35] with a little modification, after initial centrifugation at 200x g for 5 min of the semen for removal of the seminal plasma, the pellet was suspended in 8 ml of fresh Roswell Park Memorial Institute medium. Then, incubation at room temperature with gently shaker for 10 min and followed by incubation at 37°C in 5% of CO\textsuperscript{2} atmosphere for 30 min. Then, 2 ml of supernatant was transferred to a conical tube and later used for further experiments.

4.5.2. Sperm number, motility and abnormal

Isolated freshly epididymis tissue was used for the sperm number, motility, and abnormal analysis. Using a light microscope to evaluate percent of sperm motility [36,37].

4.6. Histological analysis

At the day of sacrifice, the rat’s testis collected and fixed in 10% neutral buffered formalin for 2 days. These specimens were embedded in paraffin, and sections were cut and stained with hematoxylin and eosin (H&E) and was done by Lie Pei Co., Ltd.

4.7. Statistical analysis

Data are expressed as mean ± SD (n = 6). One way and two-way ANOVA followed by Duncan’s test, respectively, were performed to compare means. P values less than 0.05 (p<0.05) was considered as significant.
5. Conclusions

The *Mytilus edulis* water extract (MWE) showed then beneficial effects to the male reproduction dysfunction in high-fat diet (HFD) induced obese rats. MWE down-regulated the plasma lipid such as triglyceride and total cholesterol. In addition, MWE also improved the enzymatic antioxidant and testosterone level as well as showed the protected effects on testicular properties. The results suggested that MWE is a potential for anti-obesity agent and it improved the testicular damage induced by obesity.

**Author Contributions:** All authors contributed to the study design and the current manuscript. Conceptualization, Zwe-Ling Kong; Formal analysis, Yi-Cheng Lu; Writing – original draft, Yi-Cheng Lu and Sabri Sudirman; Writing – review & editing, Sabri Sudirman and Chien-Feng Mao.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**References**


