Effect of Melatonin, Vitamin C and Zinc Alone or in Combination on Lipid Fraction and Histological Structure of Liver in Obese Rats

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ABSTRACT

This study was undertaken to determine the effect of two levels of melatonin, vitamin C and zinc alone or in combination on lipid fractions including (cholesterol, triglycerides, high density lipoprotein-cholesterol HDL-c, low and very low density lipoprotein-cholesterol LDL-c and VLDL-c) and histological changes of liver in obese rats. Two main experimental groups used in this experiment. The first main group (6 rats) fed on basal diet as a control negative, while the second main group (54 rats) was fed on high fat diet (HFD) 6 week to induce obesity in rats. Then, the second main group divided into nine subgroups. Subgroup (1) (6 rats) fed on HFD as positive control group. Subgroups (2 and 3) were fed on (HFD) and treated with (5 and 10 mg melatonin / kg B.W), respectively. Subgroups (4 and 5) were fed on (HFD) and treated with (100 and 200 mg vitamin C/kg B.W), respectively. Subgroups (6 and 7) were fed on (HFD) and treated with (200 and 300 mg Zn/kg diet), respectively. Subgroups (8 and 9) were fed on (HFD) and treated with (5 mg melatonin + 100 mg vitamin C + 200 mg Zn) and (10 mg melatonin + 200 mg vitamin C + 300 mg Zn), respectively.

Feeding obese rats on high fat diet caused significant increase in serum total cholesterol, triglycerides, LDL-c and VLDL-c, while HDL-c decreased significantly, as compared to healthy rats fed on basal diet (control negative group), on the other hand all tested groups showed improvement in these parameters, spatially the obese groups that treated with high and low levels from the combination of melatonin, vitamin C and zinc, followed by the group which treated with 300 mg zinc, respectively.

With regard to the histopathological examination of liver, all tested groups showed improvement in liver structure, spatially the groups treated with 200 mg Vitamin C/kg B.W; 200 mg Zn/kg diet and the groups treated with high and low levels from the combination of melatonin, vitamin C and zinc. It was concluded that melatonin, vitamin C and zinc or their combination should be used to improve the lipid fractions in obese rats.

Key words: Obesity, Rats, Melatonin, Vitamin C, Zinc, Glucose, Lipid profile, Liver and Histopathology.

Introduction

Obesity results in increase of adipocyte size (hypertrophy) and adipose tissue ds-function, creating an imbalance of Pro- and anti-inflammatory factors, which can alter the immune response and inflammatory status both locally and systemically. For example increase of fat mass and larger adipocytes lead to elevate circulating leptin concentration and leptin, in turn, exert pro inflammatory actions by activating various immune cells. Six of eight studies that distinguished between males and females found a significantly higher prevalence of overweight / obesity in females than in males in Algeria, Egypt, Morocco, Tunisia and Sudan. The obesity prevalence ranged from 56% in men with higher socioeconomic status in urban Egypt to 6% in men in rural Egypt. Higher socioeconomic status was associated with decreased physical activity and increased prevalence of obesity (Fernandez - Riejos et al. 2010).

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Worldwide obesity was more than doubled since 1980. In 2014, more than 1.9 billion adults, 18 years and older, were overweight, of these over 600 million were obese. Overall, about 13% of the world’s adult population (11% of men and 15% of women) were obese. In 2014, 39% of adults aged 18 years and over (38% of men and 40% of women) were overweight. The worldwide prevalence of obesity was more than doubled between 1980 and 2014. In 2014, an estimated 41 million children under the age of 5 years were overweight or obese. In developing countries with emerging economies (classified by the World Bank as lower – and middle – income countries) the rate of increase of childhood overweight and obesity has been more than 30% higher than that of developed countries (WHO, 2015).

Obesity is directly or indirectly associated with a variety of health disorders including hypertension, cardiovascular disease, diabetes, stroke, arthritis, immobility, cancer and premature death (WHO, 2000).

It is obvious that the current strategies are used to curb the frequency of obesity lack the desired outcome. To identify alternative strategies or approaches to retard the increased tendency towards obesity provides an urgent agenda for scientists. A new expectation for curbing obesity has emerged for recent studies that show that functional and substantial amounts of brown adipose tissue (BAT) persist in adult humans (Enerback, 2009).

Melatonin known chemically as N-acetyl -5-methoxytryptamine, is a hormone found in animals, plants and microbes (Caniato et al., 2003 and Paredes et al., 2009). In animals, circulating levels of melatonin vary in a daily cycle, thereby allowing the entrainment of the circadian rhythms of several biological functions (Altun and Ugur, 2007).

Kaur and Ling, (2008) reported that melatonin, produced in the pineal gland which is outside of the blood-brain barrier, acts as an endocrine hormone since it is released into the blood. In humans, melatonin is produced by the Pineal gland, a small endocrine gland located in the center of the brain but outside the blood-brain barrier. The melatonin signal forms part of the system that regulate the sleep –wake cycle by chemically causing drowsiness and lowering the body temperature, but it is the central nervous system (specifically the suprachiasmatic nuclei, that controls the daily cycle in most components of the paracrine and endocrine systems. In humans, 90% of melatonin is cleared in a single passage through the liver, a small amount is excreted in urine, and a small amount is found in saliva (Buscemi et al., 2004).

Many biological effects of melatonin are produced through activation of melatonin receptors, while others are due to its role a pervasive and powerful antioxidant, with a particular role in the protection of nuclear and mitochondria and DNA (Reiter et al., 2001).

Tan et al., (1993) cleared that besides melatonin function as synchronizer of the biological clock, melatonin is a powerful free-radical scavenger and wide-spectrum antioxidant as discovered in 1993. Melatonin is an antioxidant can easily cross cell membranes and the blood-brain barrier (Tan et al., 2007). This antioxidant is a direct scavenger of radical oxygen and nitrogen species (Poeggeler et al., 1994).

Melatonin works with other antioxidants to improve the overall effectiveness of each antioxidant. Melatonin has been proven twice the active as vitamin E, believed to be the most effective lipophilic antioxidant (Arnao and Ruiz, 2006).

Melatonin is involved in energy metabolism and body weight control in small animals. Many studies show that chromic melatonin supplementation in drinking water reduce body weight and abdominal fat in experimental animals, especially in the middle-aged rats (Wolden et al., 2000).

Tan et al., (2011) reported that melatonin supplementation in drinking water reduces body weight and did not require the animals to eat less and to be physically more active. A potential mechanism is that melatonin promotes the recruitment of brown adipose tissue (BAT) as well as enhances its activity. This effect would rise the basal metabolic rate by stimulating thermogenesis, heat generation through uncoupling oxidative phosphorylation in mitochondria.

Vitamin C is required for the prevention of scurvy, and in addition to its powerful antioxidant activity, it plays an important role as a cofactor in enzymes activation and immune function. Vitamin C has anti-inflammatory effects, prevents endothelial dysfunction and apoptosis, and reduces the risk of arteriosclerosis, cardiovascular disease, obesity and some forms of cancer (Kensara, 2013).
Johnston (2005) reported that Individuals with adequate vitamin C status oxidize 30% more fat during a moderate exercise bout than individuals with low vitamin C status; thus, vitamin C depleted individuals may be more resistant to fat mass loss.

Survey data suggested an inverse relationship between vitamin C status and body weight and waist measurements. The underlying systemic oxidative stress associated with obesity has been proposed to explain the inverse relation between adiposity and plasma vitamin C concentrations (Canoy et al., 2005).

Since vitamin C is an essential cofactor for the biosynthesis of carnitine, a molecule required for the oxidation of fatty acids. A reduction in the ability to oxidize fat may contribute to the relationship between vitamin C status and adiposity (Hoppel, 2003 and Reda et al., 2003).

Gaur and Dixit (2011) reported that supplementation with 500 mg vitamin C decreases cholesterol and LDL-C and improves lipid profiles. However, have no statistically significant effect on VLDL-C, HDL-C and triglycerides. The study suggests the need for a prolonged vitamin C supplementation, which is also an important constituent of the antioxidant system.

Garcia-Diaz et al., (2014) mentioned that vitamin C intake (ascorbic acid) is negatively associated with the occurrence of several conditions such as hypertension, gallbladder disease, stroke, cancers, and atherosclerosis, and also with the onset of obesity in humans and animals. Among the possible beneficial effects of ascorbic acid on obesity-related mechanisms, it has been suggested that this vitamin may: (a) modulate adipocyte lipolysis; (b) regulate the glucocorticoid release from adrenal glands; (c) inhibit glucose metabolism and leptin secretion on isolated adipocytes; (d) lead to an improvement in hyperglycemia and decrease glycosylation in obese-diabetic models; and (e) reduce the inflammatory response. Possibly, all these features could be related with the outstanding antioxidant characteristics of this vitamin.

Zinc is a mineral that plays a vital role in many biological processes, such as enzyme action, cell membrane stabilization, gene expression and cell signaling. It is required for structural and functional integrity of more than 2000 transcription factors and 300 enzymes; hence, almost all metabolic pathways are in some ways reliant on at least one Zinc requiring protein. Zinc also plays an important role in insulin action and carbohydrate metabolism. Studies have shown that diabetes is accompanied by hypozincemia and hyperzincuria. In addition, Zinc is also an integral part of key antioxidant enzymes and Zinc deficiency impairs their synthesis, resulting in increased oxidative stress. Several human studies have demonstrated that Zinc supplementation reduces total cholesterol, LDL cholesterol and triglycerides, in addition to increasing the HDL cholesterol levels (Priyanga Ranasinghe et al., 2015).

Simon and Taylor, 2001 reported that a lower level of dietary Zn supplementation (300 mg Zn/kg diet) for 6 weeks was effective for reducing fasting hyperglycemia and hyperinsulinemia and reducing weight gain (i.e. better metabolic control) in young db/db mice.

Kadhim et al., (2006) reported that the combination of melatonin (10 mg) and zinc acetate (50 mg) when used alone or in combination with metformin at bedtime for 90 days improves diabetes mellitus (2 DM)-related complications such as the impaired lipid profile and micro albuminuria in type 2 D Patients.

Therefore, the present study aims to investigate the effect of melatonin, vitamin c and zinc alone or in combination on lipid fractions and histological structure of liver in obese rats.

Materials and Methods

Materials:

1. Casein, vitamins, minerals, cellulose, choline chloride were purchased from El-Gomhoria Company, Cairo, Egypt.
2. Starch and corn oil was obtained from local market in Cairo, Egypt.
3. Melatonin was obtained from International Scientific Company and Medical Supplies, Cairo, Egypt.
4. Kits for biochemical analysis was obtained from Alkan for Pharmaceutical and Chemical, Dokki, Egypt.
5. Adult male albino rats Sprague Dawley Strain (n = 60 rat) weighing (200 ± 10 g) were purchased from Helwan farm of experimental animals, Ministry of Health and Population, Helwan, Cairo, Egypt.

Methods:

Biological investigation:

Sixty adult male albino rats of Sprague Dawley Strain weighing approximately (200 ± 10 g) were housed in well-aerated cages under hygienic condition and fed on basal diet (BD) for one week for adaptation according to (Reeves et al., 1993). After adaptation period, the rats were divided into two main groups as follows: The first main group (6 rats) was fed on basal diet (BD) as a control negative group (-ve). The second main group (54 rats) was fed on high fat diet 6 week, containing (saturated fat 19%, corn oil 1% to provide essential fatty acids, sucrose 10%, casein 20%, cellulose 5%, vitamin mixture 1%, salt mixture 3.5%, choline chloride 0.25% and the remainder is corn starch) to induce obesity in rats (Min et al., 2004).

After these periods, the mean value of body weight % was estimated in the two main groups, also blood samples were collected from all rats to estimate the levels of cholesterol and triglycerides (healthy rats was 75.640 ± 4.098 mg/dl cholesterol and 35.765 ± 4.807 mg/dl triglycerides), while the second main group recorded (160.232 ± 5.652 mg/dl cholesterol and 70.481 ± 5.032 mg/dl triglycerides), then the rats were divided into nine subgroups (n = 6 each) according to the following scheme:

Subgroup (1): was fed on high fat diet (HFD) as a control positive group (+Ve). Subgroups (2 and 3): were fed on (HFD) and treated with (5 and 10 mg melatonin / kg B.W), respectively. Subgroup (4 and 5): were fed on (HFD) and treated with (100 and 200 mg vitamin C/kg B.W), respectively. Subgroup (6 and 7): were fed on (HFD) and treated with (200 and 300 mg Zn/kg diet), respectively. Subgroup (8 and 9): were fed on (HFD) and treated with (5 mg melatonin + 100 mg vitamin C + 200 mg Zn) and (10 mg melatonin + 200 mg vitamin C + 300 mg Zn), respectively.

At the end of the experimental period (8 weeks), rats were fasted over night before sacrificing. Blood samples were collected from each rat and centrifuged at 3000 r.p.m. to separate the serum. Serum was carefully separated and transferred into dry clean Ebendorf tubes and kept frozen at -20 °C until analysis.

Liver of rats were removed by careful dissection and blotted free of adhering blood immediately after sacrificing the rats. The organs were washed with cold saline and dried between two filter papers, then weighed and kept in formalin solution (10 %) according to Drury and Wallington (1980).

Biochemical Analysis of Serum:

Total cholesterol in the serum was determined according to the method described by Allain and Poon (1974). Triglycerides were determined according to the method described by (Fossati and Principle, 1982). High density lipoprotein- cholesterol (HDL-c) was determined according to the method described by (Burstein, 1970). Low density lipoprotein-cholesterol (LDL-c) was determined according to the method described by (Friedwald et al., 1972) and very low density lipoprotein-cholesterol (VLDL-c) was estimated according to the method described by (Friedwald et al., 1972).

Histopathological Examination: Specimens from Liver tissues were taken immediately after sacrificing animals, and fixed in 10% buffered neutral formalin solution. The fixed specimens were then trimmed, washed, dehydrated and imbedded in paraffin, cut in sections of 46 microns thickness and stained with haematoxylin and eosin stain according to (Sheehan and Hrapchak, 1980).

Statistical Analysis: The data obtained was analyzed statistically for standard deviation and one-way ANOVA test (Steel and Torri, 1980).
Results and Discussion

Effect of high fat diet with or without melatonin administration, vitamin C and zinc supplementation on serum total cholesterol and triglycerides in obese rats:

Results presented in Table (1) illustrate the effect of high fat diet HFD with or without melatonin administration, vitamin C, and zinc supplementation on serum total cholesterol (TC) and triglycerides (TG) as mg/dl of obese albino rats. Results revealed that the (TC) and (TG) in control (-ve) and control (+ve) groups were 78.793 ± 3.923 and 40.002 ± 2.094 vs 176.291 ± 4.184 and 86.960 ± 3.474, respectively.

Statistical analysis showed a significant increase (P<0.05) in (TC and TG) levels in high fat diet (+ve) control group, as compared to the (-ve) control group which fed on basal diet BD.

On the other side, results revealed that obese groups which fed on HFD with melatonin administration or supplemented with vitamin C or zinc and combination between them at low or high levels recorded TC levels (156.244 ± 4.464, 131.674 ± 5.259, 148.024 ± 7.216, 134.475 ± 4.587, 150.229 ± 5.446, 138.475 ± 4.800, 134.017 ± 3.657 and 12.101 ± 4.993; respectively). While serum level of TG recorded (74.142 ± 3.413, 56.965 ± 4.013, 71.283 ± 0.994, 60.223 ± 4.094, 73.243 ± 2.961, 62.648 ± 3.350, 64.483 ± 5.230 and 50.710 ± 1.988); respectively.

Statistically, results showed a significant decrease (P<0.05) in serum TC and TG as compared with the (+ve) control group in all obese rats fed on HFD with or without melatonin administration, vitamin C, zinc or combination between them at low or high levels. The best results recorded by (+ve) group fed on HFD with melatonin administration at high level plus combination from high dose of vitamin C and zinc followed by groups fed on HFD with melatonin administration at high level. Then the group, which was fed by HFD supplemented with high level from vitamin C and the group, which was fed on HFD with low level of melatonin administration plus vitamin C and zinc supplementation.

Table 1: Effect of high fat diet with or without melatonin administration, vitamin C and zinc supplementation on serum total cholesterol and triglycerides in obese rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
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<tbody>
<tr>
<td>Control negative (-ve) fed on basal diet (BD)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>78.793 ± 3.923</td>
<td>40.002 ± 2.094</td>
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<tr>
<td>Control positive (ve+) fed on (HFD)</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>176.291 ± 4.184</td>
<td>86.960 ± 3.474</td>
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<tr>
<td>Fed on (HFD) + 5mg melatonin</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>156.244 ± 4.464</td>
<td>74.142 ± 3.413</td>
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<tr>
<td>Fed on (HFD) + 10mg melatonin</td>
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<tr>
<td></td>
<td>131.674 ± 5.259</td>
<td>56.965 ± 4.013</td>
</tr>
<tr>
<td>Fed on (HFD) + 100mg vitamin C</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>148.024 ± 7.216</td>
<td>71.283 ± 0.994</td>
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<tr>
<td>Fed on (HFD) + 200mg vitamin C</td>
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<tr>
<td></td>
<td>134.475 ± 4.587</td>
<td>60.223 ± 4.094</td>
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<tr>
<td>Fed on (HFD) + 200mg zinc</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>150.229 ± 5.446</td>
<td>73.243 ± 2.961</td>
</tr>
<tr>
<td>Fed on (HFD) + 300mg zinc</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>138.475 ± 4.800</td>
<td>62.648 ± 3.350</td>
</tr>
<tr>
<td>Fed on (HFD) + 5mg melatonin + 100mg vitamin C +</td>
<td></td>
<td></td>
</tr>
<tr>
<td>200mg zinc</td>
<td>134.017 ± 3.657</td>
<td>64.483 ± 5.230</td>
</tr>
<tr>
<td>Fed on (HFD) + 10mg melatonin + 200mg vitamin C</td>
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<tr>
<td>200mg zinc</td>
<td>112.101 ± 4.993</td>
<td>50.710 ± 1.988</td>
</tr>
</tbody>
</table>

- HFD: High Fat Diet
- All results are expressed as mean ± SD
- Values in each column, which have different litters, are significantly different (P< 0.05)
These findings are in agreement with Prunet-Marcassus et al. (2003) who demonstrated that melatonin may act as a regulator of body weight in a model of obesity and may prevent some of the side effects on glucose homeostasis such as decreases of plasma glucose, leptin and triglyceride levels.

The present results are in agreement with Kadhim et al. (2006) who reported that, the combination of melatonin and zinc, when used alone or in combination with metformin improves type 2 diabetes mellitus complications such as the impaired lipid profile, fasting lipid profiles and microalbuminuria (MAU).

The present results agreed with Tan et al. (2011) who found that the physiology of BAT regulated by melatonin, which not only increases recruitment of brown adipocytes but also elevates their metabolic activity in mammals. Melatonin administration ameliorates over weight and lipid metabolism in humans; melatonin might also help to prevent cardiovascular disease associated with obesity and dislipidemia (Agil et al., 2011).

**Effect of high fat diet with or without melatonin administration, vitamin C and zinc supplementation on serum lipid fraction in obese rats:**

Results in Table (2) illustrate the effect of high fat diet with or without melatonin administration, vitamin C and zinc supplementation on serum lipid fraction of obese rats. Results revealed that HDL-C level of (+ve) control group fed on HFD and (-ve) control group fed on BD recorded 21.614 ± 1.969 VS 49.228 ± 2.571; respectively. While LDL-C and VLDL-C values recorded 137.285 ± 2.417 and 17.392 ± 0.694 vs 21.564 ± 2.216 and 8.000 ± 0.418; respectively. Statistically, there is a significant increase (P<0.05) in HDL-C and VLDL-C; in contrast, a significant decrease (P<0.05) in HDL-C in the (+ve) control group as compared to the (ve-) control group fed on BD.

**Table 2:** Effect of high fat diet with or without melatonin administration, vitamin C and zinc supplementation on serum lipid fraction in obese rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>HDL-c mg/dl</th>
<th>LDL-c mg/dl</th>
<th>VLDL-c mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control negative (-ve) fed on basal diet (BD)</td>
<td>49.228±2.571</td>
<td>21.564±2.216</td>
<td>8.000±0.418</td>
<td></td>
</tr>
<tr>
<td>Control positive (ve+) fed on (HFD)</td>
<td>21.614±1.969</td>
<td>137.285±2.417</td>
<td>17.392±0.694</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 5mg melatonin</td>
<td>30.976±1.604</td>
<td>110.439±2.455</td>
<td>14.828±0.682</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 10mg melatonin</td>
<td>36.971±2.386</td>
<td>83.310±2.883</td>
<td>11.392±0.802</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 100mg vitamin C</td>
<td>35.196±1.921</td>
<td>98.572±5.186</td>
<td>14.256±0.198</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 200mg vitamin C</td>
<td>39.345±2.073</td>
<td>83.085±2.367</td>
<td>12.844±0.818</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 200mg zinc</td>
<td>33.104±1.727</td>
<td>102.476±4.828</td>
<td>14.648±0.592</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 300mg zinc</td>
<td>37.130±1.645</td>
<td>87.676±1.855</td>
<td>12.529±0.670</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 5mg melatonin + 100mg vitamin C + 200mg zinc</td>
<td>38.269±2.844</td>
<td>83.990±1.525</td>
<td>12.896±1.046</td>
<td></td>
</tr>
<tr>
<td>Fed on (HFD) + 10mg melatonin + 200mg vitamin C + 300mg zinc</td>
<td>41.322±1.518</td>
<td>60.637±3.204</td>
<td>10.141±0.397</td>
<td></td>
</tr>
</tbody>
</table>

- HFD: High Fat Diet
- All results are expressed as mean ± SD
- Values in each column, which have different litters, are significantly different (P< 0.05).

Concerning (+ve) groups which were fed on HFD with low or high levels of melatonin administration with or without vitamin C or Zinc supplementation results of HDL-C level recorded (30.976±1.604, 36.971 ± 2.386, 35.196 ± 1.921, 39.345 ± 2.073, 33.104 ± 1.727, 37.130 ± 1.645, 38.269 ± 2.844 and 41.322 ± 1.518); respectively. These results reveal a significant increase (P<0.05) in HDL-C as compared to the (+ve) control group. While LDL-C and VLDL-C recorded (110.439±
Results of the present study of all treatments recorded a significant decrease (P<0.05) in LDL-C and VLDL-C, levels as compared to the (+ve) control group. These findings agree with that obtained by Tan et al. (2002) who reported that combinations of melatonin with other antioxidant clearly increase their efficiency. The mechanism of the synergy remains unknown and confirmation of these findings, melatonin has proven superior to vitamins C and E in reducing oxidative damage. Present results are in agreement with Dauchy (2003) who suggested that a novel role of melatonin in the regulation of fatty acids transport and fat metabolism in skeletal muscle.

The combination of melatonin and zinc acetate, when used alone or in combination with metformin, improve type 2 DM related complication such as the impaired lipid profile (kadhim et al., 2006).

Results of this study are also at the same line with Newsom et al. (2008) who reported that acute administration of ascorbic acid, a potent antioxidant, prevented the increase in plasma concentration of oxidized low-density lipoprotein.

**Histopathological Examination of Liver:**

Microscopically, liver of rat from group1 (Negative Control) revealed normal histological structure of hepatic lobule as shown in (Photo. 1). In obese rats which fed on high fat diet with or without melatonin administration, vitamin c and zinc supplementation, our histopathological examination showed that liver of rat from group 2 (Positive Control) fed on HFD without treatment revealed steatosis of hepatocytes (Photo. 2), cystic dilatation of bile duct and fibroplasia in portal triad (Photo. 3). In group of obese rats fed on HFD with melatonin administration at low level group 3 showed vacuolization of focal hepatocytes (Photo. 4). Meanwhile (Photo. 5) showed the group 4 which fed on HFD with melatonin administration at high level showing slight vacuolation of hepatocytes. Photo.(6 and 7) showed the effect of HFD supplementation with 100mg /kg B.W. or 200mg /kg B.W. of vitamin C on liver histopathological change. Photo.(6 and 7) revealed that at low level of vitamin C group 5 (Photo. 6) showing slight vacuolation of hepatocytes, while high level of vitamin C group 6 (Photo. 7) showing no histopathological changes. However, liver of rats from groups 7, 8, some examined sections from group 9 and group 10 showed no histopathological changes (Photo. 8, 9, 10 and 11).

**Photo. 1:** C.S. in liver of rat from group 1 (Negative Control) showing the normal histological structure of hepatic lobule (H & E X 400).
Photo. 2: C.S. in liver of rat from group 2 (Positive Control) showing steatosis of hepatocytes (H & E X 400).

Photo. 3: C.S. in liver of rat from group 2 (Positive Control) showing cystic dilatation of bile duct and fibroplasia in portal triad (H & E X 400).
**Photo. 4:** C.S. in liver of rat from group 3 (Rats Fed on HFD and Treated with 5 mg Melatonin / kg B.W.) showing vacuolization of focal hepatocytes (H & E X 400).

**Photo. 5:** C.S. in liver of rat from group 4 (Rats Fed on HFD and Treated with 10 mg Melatonin / kg B.W.) showing slight vacuolation of hepatocytes (H & E X 400).
Photo. 6: C.S. in liver of rat from group 5 (Rats Fed on HFD and Treated with 100 mg Vitamin C/kg B.W.) showing slight vacuolation of hepatocytes (H & E X 400).

Photo. 7: C.S. in liver of rat from group 6 (Rats Fed on HFD and Treated with 200 mg Vitamin C/kg B.W.) showing no histopathological changes (H & E X 400).
Photo. 8: C.S. in liver of rat from group 7 (Rats Fed on HFD and Treated with 200 mg Zn/kg Diet) showing no histopathological changes (H & E X 400).

Photo. 9: C.S. in liver of rat from group 8 (Rats Fed on HFD and Treated with 300 mg Zn/kg Diet) showing no histopathological changes (H & E X 400).
Photo. 10: C.S. in liver of rat from group 9 (Rats Fed on HFD and Treated with 5 mg Melatonin + 100 mg Vitamin C + 200 mg Zn) showing no histopathological changes (H & E X 400).

Photo. 11: C.S. in liver of rat from group 10 (Rats Fed on HFD and Treated with 10 mg Melatonin + 200 mg Vitamin C + 300 mg Zn) showing no histopathological changes (H & E X 400).
References


