**Abstract.** – OBJECTIVE: Metabolic syndrome (MetS) can be induced by the oxidative stress conditions caused by ingestion of large amounts of fructose. We investigated the possible protective effects of melatonin administration on liver tissues in fructose-fed rats.

MATERIALS AND METHODS: Thirty-two rats were randomly divided into four groups; control, fructose, melatonin, and fructose plus melatonin. MetS was induced by a fructose solution (20% in tap water) and melatonin (20 mg/kg daily) was administered by oral gavage. Systolic blood pressures (SBP) were measured. After the end of the 8-week experimental period, serum lipid profile, glucose and insulin levels, tissue total oxidant status (TOS) and activities of paraoxonase (PON), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) were measured.

RESULTS: Fructose consumption significantly increased SBP, serum triglyceride and insulin levels and induced insulin resistance, confirming successful establishment of the MetS model. After fructose administration, the TOS levels and GSH-Px activities significantly increased in all groups compared to the control group. The PON activity in the fructose group significantly decreased compared to the control group. Melatonin supplementation, with or without fructose, increased PON activity. The SOD activity significantly increased in the fructose group compared to the control group, but significantly decreased in the melatonin group compared to the control and fructose groups. CAT activity was unchanged in all groups.

CONCLUSIONS: GSH-PX and PON are important antioxidants for reducing oxidant stress. Melatonin might act as a prooxidant at the dose given in our experimental design when administered with fructose.

**Key Words:** Melatonin, Metabolic syndrome, Fructose, Oxidative stress, Liver.

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**Introduction**

Metabolic syndrome (MetS) is an endocrinopathy associated with a deadly combination of systemic disorders including abdominal obesity, insulin resistance, glucose intolerance, dyslipidaemia, hypertension, and coronary artery disease. Recent reports have shown that excessive fructose intake induces features of MetS in both obese and non-obese humans. Fructose is an important nutritional factor in the development of MetS in humans, and high fructose intake in rats leads to development of MetS-associated symptoms. The most important sources of dietary fructose are prepared foods containing fructose or high fructose corn syrup as sweeteners, where fructose can occur occurring at ratios of 55-90%.

MetS is often characterized by oxidative stress and inflammation, and increased oxidative stress has an important influence on the pathogenesis of MetS. Elevated levels of oxidative stress in subjects with MetS have been demonstrated in many experimental and clinical studies. In particular, an impaired balance exists between the prooxidant/antioxidant system that favours the prooxidant system, resulting in low-grade inflammation, endothelial dysfunction and insulin resistance. The defence mechanisms of the body against oxidative stress are complex and involve cellular and extracellular antioxidant systems, which are regulated at multiple levels by several enzymes, including paraoxonase (PON), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), and catalase (CAT).

PON is a glycoprotein synthesized primarily by the liver and associated with HDL. One of its roles is to protect LDL from oxidation. Decreases in PON activity in humans and experimental animals are associated with the develop-
ment atherosclerosis and insulin resistance and increases in oxidative stress\textsuperscript{16,17}. PON activity also shows a close relationship with the activity of other antioxidant enzymes. Various \textit{in vitro} and \textit{in vivo} studies in animals and humans have provided initial evidence that antioxidants can increase PON activity, possibly by protecting the enzyme from inactivation induced by oxidative stress\textsuperscript{18}.

Melatonin (N-acetyl-5-methoxytryptainine), a derivative of indoleamine, is primarily secreted from the pineal gland, with lesser amounts from the retina, lens, neurons, airway epithelium, bone marrow, platelets, lymphocytes, placenta, intestine, testis, ovary and skin cells. Melatonin synthesis in pineocytes is triggered by epinephrine and norepinephrine from sympathetic axon endings. Melatonin is secreted into the blood, but its half-life is only about ten minutes as it is immediately metabolized by the liver. Melatonin that is synthesized outside the pineal gland does not show a circadian rhythm and appears to function essentially as an antioxidant to reduce the effects of oxidative stress.

Melatonin can also affect transcription factors to decrease the synthesis of inflammatory mediators. The target tissues of melatonin include almost all tissues and cells. Unlike other hormones, melatonin receptors are located both at the cell surface and within the cell\textsuperscript{11}. Clinical studies on patients with essential hypertension and diabetes have demonstrated that melatonin can reduce blood pressure\textsuperscript{19,20}. Melatonin treatment also ameliorates metabolic changes associated with obesity in rats fed a high-fat diet\textsuperscript{1,21,22}. Leibowitz et al\textsuperscript{23} showed that melatonin secretion was decreased during sleep in rats fed a diet containing 60% fructose and that five weeks of daily supplementation of melatonin in the drinking water of these rats suppressed hypertension without attenuating the increased serum triglyceride level. Increasing evidence also indicates that melatonin lowers oxidative stress in humans\textsuperscript{24-26}. However, a role for melatonin in improving MetS induced by high fructose intake has not been established.

The aim of this study was, therefore, to evaluate the effect of melatonin on oxidative stress and the antioxidative system in a MetS model of rat liver. The rates were fed 20% fructose in the drinking water for 8 weeks and we measured total oxidant status (TOS) and the activities of the antioxidative enzymes SOD, CAT, GSH-PX, PON in the liver.

### Materials and Methods

**Chemicals**

Melatonin (\(\geq 98\%\)) and D-Fructose (\(\geq 99\%\)) were purchased from Sigma-Aldrich (St. Louis MO, USA). All other chemicals used were of the highest analytical grade, and purchased from Merck or Sigma-Aldrich.

**Animals and Experimental Design**

This study was carried out in accordance with the regulations of Animal Experimentation Ethics Committee of Gazi University. Thirty-two adult male Sprague-Dawley rats weighing 225±10 g were obtained from Laboratory Animal Husbandry and Experimental Research Center at Gazi University. The animals were housed at 20-24°C with a 12-h light, 12-h dark cycle (the lights were off daily from 20.00 through 08.00 h) and they were provided with standard rat chow and tap water freely available. Thirty-two rats were randomly divided into four groups (\(n = 8\)) as follows;

1. **Control**: Rats in this group received standard rodent diet and tap water.
2. **Fructose**: Rats in this group received standard rodent diet and tap water supplemented with 20% fructose\textsuperscript{27}.
3. **Fructose plus Melatonin**: Rats in this group received standard rodent diet and tap water supplemented with 20% fructose, and melatonin administered at the dose of 20 mg/kg body weight in 0.1% ethanol solution per day by oral gavage.
4. **Melatonin**: Rats in this group received standard rodent diet and tap water, and melatonin administered at the dose of 20 mg/kg body weight in 0.1% ethanol solution per day by oral gavage\textsuperscript{23}. Melatonin solution was prepared freshly every day.

Since ethanol was used as a melatonin’s vehicle, control and fructose groups received 0.1% ethanol solution proportionately with body weight. The experiment was carried out for 8 weeks and at the end of the 8\textsuperscript{th} week, the animals were sacrificed under ketamine (30 mg/kg bw) and xylazine (6 mg/kg bw) anesthesia. Blood samples for laboratory assays were drawn intracardially and sera were separated by centrifugation. Sera and liver tissues were stored at –80°C until analysis.

**Systolic Blood Pressure, Body Weight, Fluid Intake**

Systolic blood pressures (SBP) were measured by tail-cuff method at the beginning of the study,
at the end of the 4th week and at the end of the 8th week. All animals were preconditioned for blood pressure measurements 1 week before each experiment. At least seven determinations were made in every session and the mean of the lowest three values within 5 mmHg was taken as the SBP value. Body weights were recorded weekly, fluid intake of all above groups was measured daily.

**Serum Analysis**

Serum glucose, triglyceride, VLDL-cholesterol levels were measured by enzymatic methods using autoanalyzers. Serum insulin level was estimated by using commercially available ELISA kit (Millipore). Insulin resistance was evaluated by the Homeostasis Model Assessment index (HOMA-IR) using the formula: \[\text{insulin (mU/L) x glucose (mmol/L)/22.5.}\]

**Liver Total Oxidant Status level and Paraoxonase Activity Measurement**

TOS level and PON activities were detected using a commercial colorimetric kit (RelAssay Diagnostics, Graziantep, Turkey) according to the manufacturer’s guidelines. The concentrations of oxidant capacity were calculated from a standard curve and expressed as \(\mu\)mol H\(_2\)O\(_2\) Equiv/g tissue and PON activities were expressed U/g tissue.

**Liver Glutathione Peroxidase Activity Measurement**

Assay for GSH-Px activities were detected in a 96-well microliter plate using Cayman colorimetric Assay Kit (Ann Arbor, MI, USA). Results were expressed as nmol/min/g tissue.

**Liver SOD Activity Measurement**

Assay for SOD activity was assayed according to the method of Yi-Sun et al\(^{(28)}\). This method employs xanthine and xanthine oxidase to generate superoxide anion which reacts with nitroblue tetrazolium (NBT) and then measured by the degree of inhibition of the reaction one unit of enzyme provides 50% inhibition of NBT reduction. Results were expressed as IU/g tissue.

**Catalase Activity Measurement**

Liver catalase activity was assayed according to the method of Aebi et al\(^{(29)}\). Briefly, H\(_2\)O\(_2\) was used as the substrate and the decrease of H\(_2\)O\(_2\) concentration at 20°C in phosphate buffer was assayed by spectrophotometry at 240 nm. One unit of catalase activity is the amount of enzyme that degrades 1 \(\mu\)mol of H\(_2\)O\(_2\)/min; catalase activity was expressed as IU/g protein.

**Statistical Analysis**

Statistical analysis of the results was conducted using Statistical Package for the Social Science (SPSS 16.0) software (SPSS Inc., Chicago, IL, USA). All values were presented as mean ± SD. In the assessment, the Kruskal-Wallis analysis of variance and to detect differences between groups, the Mann-Whitney U test was used. Probability values of less than 0.05 were accepted as statistically significant.

**Results**

**Metabolic Syndrome Criteria**

Fructose administration caused a significant increase in SBP, serum triglycerides, VLDL-cholesterol and insulin and it induced insulin resistance, indicating successful establishment of the MetS rat model (Table I). Comparison of the oxidative stress parameters between the fructose administration and control groups revealed a statistically significant increase in the TOS level in the fructose group compared to the control group. By contrast, melatonin, supplied alone or in combination with fructose, did not change the TOS level when compared to the fructose group.

<table>
<thead>
<tr>
<th></th>
<th>SBP (mmHg)</th>
<th>Triglycerides (mg/dl)</th>
<th>VLDL-Cholesterol (mg/dl)</th>
<th>Insulin (mU/L)</th>
<th>HOMA-IR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>125.3 ± 1.29</td>
<td>36.00 ± 8.07</td>
<td>8.00 ± 2.39</td>
<td>4.79 ± 1.81</td>
<td>2.67 ± 1.11</td>
</tr>
<tr>
<td>Fructose</td>
<td>160.1 ± 1.40(^a)</td>
<td>93.75 ± 15.85(^c)</td>
<td>18.75 ± 3.24(^a)</td>
<td>28.21 ± 6.03(^b)</td>
<td>16.71 ± 4.89(^b)</td>
</tr>
<tr>
<td>Melatonin</td>
<td>124.1 ± 1.12(^b)</td>
<td>36.87 ± 8.76(^b)</td>
<td>7.25 ± 1.83(^b)</td>
<td>4.74 ± 1.02(^b)</td>
<td>2.18 ± 0.51(^b)</td>
</tr>
<tr>
<td>Fructose+melatonin</td>
<td>125.5 ± 1.14(^b)</td>
<td>80.75 ± 17.41(^c)</td>
<td>16.25 ± 3.53(^b)</td>
<td>14.11 ± 3.23(^b)</td>
<td>8.54 ± 3.04(^b)</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. \(^p < 0.05\) as compared to control group; \(^p < 0.05\) as compared to fructose group; \(^p < 0.05\) as compared to melatonin group.
The PON activities were statistically significantly lower in the fructose treatment group than in the control group. Melatonin supplementation, alone or in combination with fructose, increased the PON activities compared to the fructose group. The GSH-Px activities significantly increased in all groups compared to the control group. The SOD activities significantly increased in the fructose group compared to the control group, but statistically significant decreases were observed in the melatonin group compared to both the control and the fructose groups. CAT activities were unchanged in all groups compared to the control group (Table II).

### Discussion

MetS is associated with insulin resistance, hyperinsulinaemia, hypertension and hyperlipidaemia and is also a risk factor for liver inflammation and fatty liver\(^\text{30}\). Previous studies have shown that the fructose-fed rat model develops an insulin resistance syndrome with a very similar metabolic profile to the human condition\(^\text{31}\). Nevertheless, the reported metabolic alterations observed in fructose-fed rats are quite divergent among different studies. This divergence may reflect differences in study designs, which could include the strain of rat used (e.g. Wistar or Sprague-Dawley), the amount and route of fructose administration (e.g. diet or drinking water), the age of the animals and the duration of fructose administration\(^\text{27}\). In our study, we used Sprague-Dawley male rats and fructose administration was accomplished by providing 20% D-fructose in tap water, prepared daily, for 8 weeks. At the end of the study, the MetS criteria of hypertension, hyperinsulinaemia, insulin resistance and hypertriglyceridaemia were observed in the fructose-treated rats when compared to the control group (Table I).

Fructose is known to stimulate fat accumulation in the liver by concomitantly increasing fat synthesis and blocking fat oxidation, leading to hepatic steatosis. In addition, high fructose intake induces oxidative stress, mainly through disruption of the antioxidant defence system. The induction of MetS is associated with elevated oxidative stress and activation of inflammatory cytokines\(^\text{9, 10}\). The results of our report agreed with those of previous studies, as a significant increase in the TOS level occurred after 8 weeks of fructose treatment and fructose intake significantly decreased the PON activity (Table II). Demir et al\(^\text{32}\) also found higher TOS levels in human MetS patients compared to the control group, as well as a significantly reduced PON activity in same patients. Nevertheless, the results presented here for antioxidant enzyme activities were contradictory, as CAT activity was unchanged, while the SOD and GSH-PX activities significantly increased (Table II). Vavrova et al\(^\text{33}\) also reported that the alterations in antioxidant enzyme activities related to MetS were not uniform. The activity of SOD was higher in the MetS group than in the healthy subjects, whereas a decrease in CAT and PON, as well as the absence of the expected increase in GSH-PX indicated a disruption of the antioxidant defence mechanism.

Some workers have suggested that a lower PON level might contribute to a greater risk of dyslipidaemia, insulin resistance and high blood pressure, which are all considered as important components in the pathogenesis of MetS\(^\text{34}\). Senti

### Table II. Liver TOS levels and liver antioxidant enzymes activities.

<table>
<thead>
<tr>
<th>Group</th>
<th>TOS (umol H₂O₂ Equiv/g tissue)</th>
<th>PON (nmol/min/g tissue)</th>
<th>GSH-Px (nmol/min/g tissue)</th>
<th>SOD (U/g tissue)</th>
<th>CAT (U/g protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.46 ± 9</td>
<td>329.2 ± 57</td>
<td>1.90 ± 0.69</td>
<td>83 ± 1.0</td>
<td>101 ± 14</td>
</tr>
<tr>
<td>Fructose</td>
<td>75.85 ± 14(^a)</td>
<td>184 ± 43(^a)</td>
<td>2.58 ± 0.92</td>
<td>85 ± 1.7(^a)</td>
<td>98 ± 5.5</td>
</tr>
<tr>
<td>(p = 0.004)</td>
<td>(p = 0.004)</td>
<td></td>
<td>(p = 0.03)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melatonin</td>
<td>82 ± 9(^a)</td>
<td>324 ± 71(^b)</td>
<td>3.01 ± 1.32</td>
<td>79 ± 3.0(^b)</td>
<td>102 ± 7.8</td>
</tr>
<tr>
<td>(p = 0.004)</td>
<td>(p = 0.013)</td>
<td></td>
<td>(p = 0.008, 0.004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fructose + melatonin</td>
<td>75 ± 17(^a)</td>
<td>263 ± 42(^a)</td>
<td>3.70 ± 0.48(^b)</td>
<td>82 ± 4.9</td>
<td>95 ± 10</td>
</tr>
<tr>
<td>(p = 0.01)</td>
<td>(p = 0.037, 0.025)</td>
<td></td>
<td>(p = 0.004, 0.025)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SD. a: p < 0.05 as compared to control group; b: p < 0.05 as compared to fructose group; c: p < 0.05 as compared to melatonin group.
The effect of melatonin on liver on oxidant stress in fructose-mediated metabolic syndrome model

et al\textsuperscript{15} reported that a greater degree of severity of MetS is associated with a progressively worse antioxidant/oxidant balance, which is consistent with increased oxidative stress and lower antioxidant PON enzymatic capacity.

The main aim of our study was to examine the effect of melatonin on oxidative stress and the antioxidative system in a fructose-mediated MetS model. The ability of melatonin to scavenge reactive oxygen species (ROS) and reactive nitrogen species (RNS) has led to the hypothesis that the main function of melatonin is to protect living organisms from oxidative stress by increasing TOS\textsuperscript{16}. Our study demonstrated that 20 mg/kg/day melatonin supplementation, with or without fructose, increased GSH-Px and PON activities in melatonin-treated animals, whereas CAT activity was unchanged and SOD activity was decreased. On the other hand, melatonin treatment had no effect on the TOS level after fructose administration (Table II).

Although conflicting results have been presented regarding melatonin effects, our findings were in agreement with some of the published evidence. Pablo set al\textsuperscript{37} reported that GSH-Px is generally considered to be important antioxidant enzyme because it metabolizes H$_2$O$_2$ and the other hydroperoxides. Devi et al\textsuperscript{38}, however, observed that melatonin treatment restored SOD activity and produced no change in the activity of CAT, while GSH-Px increased in alloxan-treated animals. Anwer et al\textsuperscript{19} reported that liver homogenates of rats treated with melatonin showed a possible normalization of the imbalance between free radical and antioxidative systems. Kotler et al\textsuperscript{40} found that exogenously administered melatonin increased the levels of mRNA for GSH-Px in the brain. Antolin et al\textsuperscript{41} investigated the expression of antioxidant enzyme genes in animal brains and reported that melatonin supplementation considerably increased the relative mRNA levels for SOD, based on northern blotting, but the mRNA for GSH-Px showed only a slight increase when compared to the control group.

Osseni et al\textsuperscript{42} studied the effect of melatonin on intracellular reduced GSH level and ROS production in liver cell lines and showed a dual effect of melatonin that depended on its concentration and incubation time – under some conditions, melatonin could show a prooxidant effect. The production of melatonin was revealed by a stimulation of ROS production. Our results are also suggestive of a dual effect in the liver, based on comparison of the data from the melatonin-only and the melatonin-plus-fructose groups compared with the fructose-only group; that is, melatonin had no effect on fructose-mediated ROS production. In contrast, melatonin may stimulate PON and GSH-Px enzymes by its dual action. Walden et al\textsuperscript{14} also reported an increase in GSH-Px activity in the brains of rats after acute administration of melatonin, again supporting a dual action by which melatonin may provide protection to cells against oxidative damage. The specific enzyme that is induced by melatonin administration might therefore depend on the tissue and the experimental design. Ozyurt et al\textsuperscript{44} reported that melatonin an decrease in GSH-Px, SOD, nitric oxide, adenosine deaminase, xanthine oxidase activity and malondialdehyde level in rat brain in an experimental psychosis model, melatonin may reduce the oxidative damage.

In our study, we observed a statistically significant increase in PON activity when melatonin was administered, either alone or in combination with fructose (Table II). No previous studies have investigated the effect of melatonin on PON activity. We believe that our study sheds light on an area for future research. The reduction in PON activity following the metabolic abnormalities induced by fructose administration suggests that the inactivation of PON is a likely consequence of oxidative stress in MetS. Therefore, the increased PON activity after melatonin treatment may reflect an important antioxidant function that serves to protect the enzyme against oxidative stress in MetS.

Conclusions

GSH-Px and PON appear to play important roles in combatting the oxidative stress induced by fructose supplementation, while SOD and CAT may not be as important. Melatonin treatment increased the GSH-Px and PON enzyme activities, suggesting that melatonin might act as a prooxidant at the dose given here when administered with fructose. This dose-dependence on dose as well as possible experimental design effects indicate that further studies are needed using different experimental conditions.

Conflict of Interest

The Authors declare that they have no conflict of interests.
References


