Protective Effects of Agomelatin, A Melatonin Receptor Agonist, on Paracetamol Induced Nephrotoxicity

Melatonin Reseptor Agonisti Agomelatin'in Parasetamol ile İndüklenmiş Nefrotoksisitede Koruyucu Etkileri

ABSTRACT
Paracetamol may cause kidney damage when used in supratherapeutic doses. Agomelatine which is a melatonin analogue is an antidepressant drug. In the light of this information, we aimed to determine the protective effect of Agomelatine on high dose paracetamol-induced nephrotoxicity by biochemical method. In our study, 42 Sprague Dawley male rats were divided into 7 groups; Control, Paracetamol (2 g/kg), NAC+Paracetamol (140 mg/kg+2 g/kg), Agomelatine+Paracetamol (20 mg/kg+2 g/kg), Agomelatine+Paracetamol (40 mg/kg+2 g/kg), Agomelatine (40 mg/kg), NAC (140 mg/kg). Biochemical examinations were performed on tissue and blood samples obtained from all groups 24 hours after paracetamol administration. In the biochemical examination, the urea and creatinine levels were significantly increased in the group induced nephrotoxicity with paracetamol, and it was determined that serum levels of urea and creatinine decreased significantly with the administration of agomelatine according to paracetamol group. This decrease did not change in different doses of agomelatine. In the evaluations made at the tissue level, in the group induced nephrotoxicity with paracetamol, GSH and SOD levels decreased significantly and MDA level increased significantly; It was determined that GSH and SOD levels increased significantly and MDA levels decreased significantly in the groups treated with agomelatine. As a result; It has been shown biochemically that agomelatine has a therapeutic effect in preventing nephrotoxicity caused by high dose paracetamol. This result was promising that agomelatine can be used in paracetamol poisoning seen in the clinic.

Keywords: Agomelatine, kidney, nephrotoxicity, paracetamol, rat

ÖZET
Parasetamol supraterapötik dozlarda kullanıldığında böbreklerde hasara neden olabilir. Agomelatine melatonin reseptör analogu olan antidepressan etkili bir ilaçtır. Bu bilgiler içinde Agomelatin’in yüksek doz parasetamol ile oluşturulan nefrotoksisite üzerine koruyucu etkisini biyokimyasal olarak belirlemeyi amaçladık. Çalışmamızda 42 adet Sprague Dawley cinsi erkek rat 7 gruba ayrıldı; Kontrol, Parasetamol (2 g/kg), NAC+Parasetamol (140 mg/kg+2 g/kg), Agomelatine+Parasetamol (20 mg/kg+2 g/kg), Agomelatine+Parasetamol (40 mg/kg+2 g/kg), Agomelatine (40 mg/kg), NAC (140 mg/kg). Parasetamol uygulamasından 24 saat sonra tüm gruptardan alınan doku ve kan örneklerinde biyokimya-
1. INTRODUCTION

Paracetamol is widely available and used without a prescription or prescription as a single compound or in combination with other medicines. Increasing usage and being easily accessible brings with it the risk of toxicity. [1, 2] When paracetamol is used in therapeutic doses, 90-95% of the paracetamol conjugated with glucuronic acid with the help of the glucuronyl transferase enzyme in the liver, conjugated with sulforic acid (∼35%) with the help of the enzyme sulfortransferase and excreted in the urine in 24 hours. 2% of it is excreted unchanged from the urine. [3]. A small proportion of 3% of paracetamol undergoes N-hydroxylation through the hepatic cytochrome P450 enzyme system (CYP2E1 and CYP1A2 isoenzymes) and forms a highly reactive intermediate, N-acetyl-p-benzoquinonimine (NAPQI) [4, 5]. This metabolite harms by covalently binding to other intracellular proteins and is reacted with sulfhydryl groups in glutathione (GSH) in physiological conditions and excreted in the urine as mercapturic acid and cysteine conjugates [4]. However, when paracetamol is taken at high doses, this reactive product consumes hepatic GSH stores and generate liver damage [6]. Paracetamol is deacetylated in the kidneys and turns into a nephrotoxin p-aminophenol metabolite. The p-aminophenol formed in therapeutic doses is conjugated with glutathione in the liver, similar to the NAPQI metabolite, and is excreted as inactive glutathione conjugates. In addition, reactive intermediates resulting from oxidation of paracetamol by the cytochrome p450 system in the kidney cortex may cause damage to the kidney cortex [7].

Studies have shown that high-dose paracetamol causes renal cortical necrosis in humans and experimental animals. Pathways involving cytochrome p450 enzyme system, prostaglandin synthase and N-deacetylation enzyme systems play a role in renal toxicity mechanisms. The resulting cellular damage causes damage especially to the proximal tubule and slower glomerular filtration rate. As in the liver, renal microsomes oxidize paracetamol with p450 enzyme system. Acute kidney damage due to this paracetamol shows that it occurs by biochemical mechanisms as in the liver. The conditions that may affect chronic liver failure, sex and p450 enzyme system may affect renal toxicity [8].

Agomelatine is melatonin analog. Agomelatine has antagonist effect on serotonin 5-HT2C receptors and agonist effects on melatonin MT1 and MT2 receptors with higher affinity [9,10]. Melatonin shows many properties including immunomodulatory [11], oncostatic [12], antiaging [13] and antioxidant [14]. Four different mechanisms of action have been described for melatonin: (a) interacting with membrane receptors; [15] (b) binding to nuclear receptors [16]; (c) interaction with cytoplasmic proteins [17]; and antioxidant activity including radical scavenging feature [18]. In vivo studies have shown that melatonin exerts its hepatoprotective effects against paracetamol toxicity by reducing both oxidative stress and inflammation [19, 20].

In this study, the protective effect of agomelatine on paracetamol-induced kidney damage will be examined in comparison with N-acetyl cysteine (NAC). Serum urea and creatinine levels, superoxide dismutase (SOD) activity and glutathione (GSH) and malondialdehyde levels were measured in order to evaluate the protective effect of agomelatine on kidney damage.
2. MATERIALS-METHODS

2.1. Animals

In this study, a total of 42 male Sprague Dawley rats from the experimental animal laboratory within Atatürk University Experimental Research and Application Center (ATADEM). Animal experiments and procedures were performed in accordance with the national guidelines for the use and care of laboratory animals and were approved by Atatürk University’s local animal care committee (24.02.2012/B.30.2.ATA.023.85-30) and Atatürk University Institute of Health Sciences Ethics Committee (8.05.2012/2012.2.1). Rats is weighing around 240-260 grams are housed in groups of six per standard plastic cages on sawdust bedding in an air-conditioned room at 22°C under lighting controls (14-hr light/10-hr dark cycle). Standard rat chow and tap water were given ad libitum.

2.2. Chemicals

Paracetamol (Doğa İlaç Raw Materials Trade Ltd. Co.): 2 g of paracetamol was dissolved in 1% CMC (Carboxymethyl Cellulose) containing PBS (phosphate solution) and mixed at a slight temperature.

Agomelatine (VALDOXAN® 25 mg tablet, Servier): 25 mg of single tablet as dissolved in 0.9% NaCl solution.

N-Acetyl Cysteine (NAC) (Asist 200 mg capsule, Hüsnü Arsan Pharmaceuticals): 600 mg of single tablet NAC was dissolved in 0.9% NaCl solution.

Thiopental Sodium (İE ULAGAY): 50 mg / kg was given by intraperitoneally for euthanasia.

2.3. Treatment

In the study, total 42 rats were divided into 7 groups of 6 rats each, one of these groups used as control. All groups were fasted for 24 hours before the experiment. The fasted animals were get involved in the experimental protocols indicated in Table 1.

All doses of paracetamol and agomelatine used in this study were determined according to literature [21, 22] and previous animal studies [23], respectively. In treatment groups, NAC and agomelatine were administrated 1 h later paracetamol administration. At 4 hours after paracetamol application, rats allowed free access of food and water. At 24 hours after paracetamol administration, animals were euthanized by i.p injection of 50 mg/kg thiopental. Blood samples of animals in all groups were collected and their kidneys removed. The kidneys were separated for biochemical analysis and stored at -80°C. Collected blood was kept in -80°C freezer.

2.4. Biochemical Analysis

2.4.1. Analysis of Kidney Tissue

100 mg tissue of each rat was homogenized with ultra-turrax on ice in specific homogenate buffer (in appropriate buffer). It was then centrifuged according to the kit procedure.

SOD activity and MDA and GSH levels from each supernatant was measured in duplicate for each rat kidney tissues with high sensitivity kits Cayman Chemical Superoxide Dismutase Assay Kit Item Number 706002, Cell Biolabs OxiSelect™ TBARS Assay Kit (MDA Quantitation) STA-330 and Cell Biolabs OxiSelect™ Total Glutathione (GSSG/GSH) Assay Kit STA-312 ELISA respectively. In addition, all data in all kidney supernatants homogenized with the appropriate buffer are shown as mean ± standard deviation for each mg protein.

2.4.2. Protein determination

Protein concentrations were determined using the Lowry method that used commercial protein standards (Sigma Aldrich, Total protein kit-TP0300-1KT-USA).

2.4.3. Analysis in Serum

2.4.3.1. Determination of serum urea and creatinine levels

Bloods taken into anti-coagulated tube were centrifuged for 10 minutes at 4000 g and sera were separated. Separated serum samples were transferred to eppendorf tubes and placed on the instrument “Co-bas C-501” for analysis on the auto analyzer. Urea and creatinine activity were calculated automatically by the device.

2.5. Statistical analysis

Statistical analysis of biochemical studies was carried out using IBM SPSS Statistics 20 software. One-way analysis of variance (ANOVA) test and
“Duncan” technique, which is one of the post-hoc tests, were used in the analysis of parametric data. The values obtained were given as mean ± standard deviation and P values below 0.05 were considered statistically significant.

3. RESULTS

3.1. Biochemical Results

As seen in Table 2, while the mean of creatinine and urea levels in the serum of control groups were found as 0.29 ± 0.06 U / L and 47.55 ± 10.77 U / L, these levels were found as 0.56 ± 0.12 U / L and 81.15 ± 21.39 respectively in the group given 2g/kg paracetamol. Creatinine and urea levels were determined as 0.30 ± 0.04 U / L and 48.80 ± 6.81 U / L, respectively, in the positive control group where only NAC was given. The mean of creatinine and urea levels in rats given paracetamol + NAC were 0.36 ± 0.08 U/L and 57.23 ± 9.21 U/L, respectively.

In the paracetamol + 20 mg/kg Agomelatin group, creatinine and urea levels were measured as 0.38 ± 0.09 U / L and 52.21 ± 6.85 U/L, respectively; in the paracetamol + 40 mg/kg Agomelatin group was measured as 0.36 ± 0.07 U/ L and 46.48 ± 8.70 U/L, respectively.

It was observed that the creatinine value was significantly high in the paracetamol group and there was a statistically significant difference between the other groups. It was observed that the creatinine value in the Para + NAC group was lower than the paracetamol group and had approximately the same creatinine values as the Para + AGO 20 mg/kg and Para + AGO 40 mg/kg groups.

When the urea levels were analyzed, it was observed that this value was very high in the paracetamol group and there was a statistically significant difference compared to other groups. Although there was no statistically significant difference between Para + NAC, Para + AGO 20 mg/kg and Para + AGO 40 mg/kg groups, the best improvement in urea values was observed in Para + AGO 40 mg/kg group.

As seen in Table 3, while the average of SOD activity, GSH and MDA levels in kidney tissue of intact rats was 37.35 ± 10.01 U / mg protein, 2.32 ± 0.38 nmol / mg protein and 38.43 ± 23.07 µg / mg protein respectively, these levels were determined as 22.52 ± 6.14 U/mg protein, 1.69 ± 0.66 nmol/mg protein and 137.57 ± 45.26 µg/mg protein in the group that administered 2 g/kg paracetamol, respectively.

These parameters in NAC group were determined as 34.78±10.91 U/mg protein, 2.54±0.60 nmol/mg protein and 76.70±27.69 µg/mg protein, respectively. SOD activity, GSH and MDA levels in the agomelatine 40 mg / kg group were 36.49 ± 13.22 U / mg protein, 2.29 ± 0.56 nmol / mg protein and 47.71 ± 20.20 µg / mg protein, respectively. SOD activity, GSH and MDA levels in the agomelatine 40 mg / kg group were 36.49 ± 13.22 U / mg protein, 2.29 ± 0.56 nmol / mg protein and 47.71 ± 20.20 µg / mg protein, respectively.

While in the Paracetamol + NAC group, these values were measured as 34.84 ± 6.85 U / mg protein, 2.38
± 0.21 nmol/mg protein and 95.44 ± 17.97 µg / mg protein, respectively; in the Paracetamol + AGO 20 mg/kg group was determined as 38.56 ± 8.69 U / mg protein, 2.20 ± 0.36 nmol/mg protein 61.90 ± 32.40 µg/mg protein; in the Paracetamol + AGO 40 mg/kg group, it was determined as 41.90 ± 7.90 U/mg protein, 2.28 ± 0.24 nmol/mg protein, 57.20 ± 10.30 µg/mg protein.

It was observed that there was a statistically significant difference in SOD activity between paracetamol group and other groups and this value decreased significantly. No significant difference was found between Paracetamol + AGO 20 mg/kg and Paracetamol + AGO 40 mg/kg groups, but SOD activity was higher in these groups compared to Paracetamol + NAC group. In addition, it was found that SOD activity increased in these groups compared to the group treated with NAC.

GSH values measured in the paracetamol group were statistically significantly lower than the other groups. No significant difference was found between Paracetamol + AGO 20 mg/kg and Paracetamol + AGO 40 mg/kg groups, but SOD activity was higher in these groups compared to Paracetamol + NAC group. In addition, it was found that SOD activity increased in these groups compared to the group treated with NAC.

As seen in Table 3, it was observed that MDA significantly increased in paracetamol group. It was observed that MDA levels showed a significant improvement in agomelatine and NAC treatment groups. Although no statistically significant difference was observed between treatment groups with agomelatine, the highest improvement in MDA level belonged to the Paracetamol + AGO 20 mg/kg group. In addition, the improvement in terms of MDA in the group treated with NAC was significantly higher than the treatment groups with Agomelatine.

4. DISCUSSION

In this study, the effects of agomelatine, a melatonin analogue, on experimentally induced paracetamol toxicity in rat kidneys were demonstrated.

Paracetamol has a reasonable safety profile when consumed in therapeutic doses, but causes severe hepatotoxicity and nephrotoxicity when used in supratherapeutic doses. [3] NAC, the specific antidote of this drug, is used in the treatment of patients admitted to the hospital due to paracetamol toxicity. NAC shows similar effects in experimental animals. [23] In our study, it was used as a positive control group. The effects of various antioxidant agents other than NAC have been evaluated in the prevention of experimental damage caused by paracetamol. Among these, a wide range of agents can be shown such as melatonin, vitamin E, Bosentan, infliximab. Likewise, the effects of various agents such as vitamin C and ginger have been evaluated in preventing kidney tissue from paracetamol toxicity. [25-27]

Agomelatine, an antioxidant substance used in this study, is melatonin analogue. Melatonin has been reported to inhibit paracetamol-induced hepatotoxicity, including lipid peroxidation and protein oxidation, in mice. [19] Melatonin is known to increase cellular GSH concentrations in human vascular endothelial cells by inducing the c-glutamylcysteine synthetase enzyme, the speed limiting enzyme in GSH synthesis. [31] In vivo studies have shown that melatonin
exerts its hepatoprotective effects against paracetamol toxicity by reducing both oxidative stress and inflammation.[19, 20] More recently, ROS (reactive oxygen compounds) formation following lipid peroxidation occurred in hepatocytes with paracetamol administration in in vitro study with primary mouse hepatocyte culture, and pre-administration of melatonin suppressed ROS production and lipid peroxidation formation in hepatocytes [20] In our study, this nephroprotective effect may be the result of stabilization in the redox step with agomelatine and maintenance of antioxidant capacity. This may also be due to the effect of agomelatine on melatonin receptor activity.

Although it is very rare, examination of renal biopsy performed due to paracetamol toxicity in light microscopy has been observed in tubular epithelial cells in both proximal and distal parts of tubules. [29] Renal insufficiency induced by paracetamol is associated with acute tubular necrosis (ATN). Urine analysis can be used to differentiate other causes of renal failure from this condition. During ATN, urine sedimentation increased with hematuria and pyuria. In other renal toxicities, acute urinary sedimentation increases less. Urine sodium and osmolality may also provide additional information. In ATN, urine sodium level is > 20 mmol/L. In addition, serum urea and creatinine levels can be used to assess kidney damage due to paracetamol toxicity to evaluate kidney function. [9, 30]

Paracetamol is excreted through the kidneys after being metabolized in the liver. Therefore, high doses of paracetamol are known to cause damage to the kidney as well as the liver. There are many studies aimed to reduce kidney damage caused by high dose paracetamol. Çekmen et al. when they analyzed the effects of turmeric in their experiments that they caused kidney damage by giving 1000 mg/kg of paracetamol, they observed that urea and creatinine values increased compared to control in paracetamol group, whereas in paracetamol + turmeric group they approached the values in the control group [31]. In their study, where Lucas et al. examined the protective effects of ribose cysteine (RibCys) in kidney damage, it was reported that RibCys triggered GSH synthesis and prevented toxicity by binding NAPQI, a toxic metabolite of paracetamol, to the cellular proteins in the kidney by binding to GSH with a covalent bond [32] Biotransformation (oxidation, reduction, hydrolysis and conjugation) of xenobiotics takes place in kidney proximal tubules. Accordingly, it is known that damage to proximal tubule cells can change creatinine and urea levels. In our study, we observed that kidney damage in the group we administered paracetamol increased urea and creatinine levels in line with the literature, and that agomelatine administration decreased these values to values close to the control group.

Lipid peroxidation is one of the most important mechanisms causing tissue damage due to paracetamol and occurs due to free oxygen radicals. MDA is the end product of lipid peroxidation and one of the most

### Table 3. Mean SOD activity and GSH and MDA levels

<table>
<thead>
<tr>
<th></th>
<th>SOD (U/mg protein)</th>
<th>GSH (nmol/mg protein)</th>
<th>MDA (µg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>37.35±10.01b</td>
<td>2.32±0.38b</td>
<td>38.43±23.07a</td>
</tr>
<tr>
<td>AGO 40 mg/kg</td>
<td>36.49±13.22b</td>
<td>2.29±0.56b</td>
<td>47.71±20.20b</td>
</tr>
<tr>
<td>NAC</td>
<td>34.78±10.91b</td>
<td>2.54±0.60b</td>
<td>76.70±27.69c</td>
</tr>
<tr>
<td>PARACETAMOL</td>
<td>22.52±6.14b</td>
<td>1.69±0.66b</td>
<td>137.57±45.26d</td>
</tr>
<tr>
<td>PARACETAMOL+NAC</td>
<td>34.84±6.85b</td>
<td>2.38±0.21b</td>
<td>95.44±17.97b</td>
</tr>
<tr>
<td>PARACETAMOL+AGO 20 mg/kg</td>
<td>38.56±8.69b</td>
<td>2.20±0.36b</td>
<td>61.90±32.40b</td>
</tr>
<tr>
<td>PARACETAMOL+AGO 40 mg/kg</td>
<td>41.90±7.90b</td>
<td>2.28±0.24b</td>
<td>57.20±10.30b</td>
</tr>
</tbody>
</table>

***AGO: Agomelatine, Statistics were evaluated with Duncan technique in One-Way ANOVA test. a, b, c, d Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups according to the Duncan test. P <0.05 was considered significant. (Values: Mean ± SD)
widely used determinants of lipid peroxidation. Tissues exposed to oxidative stress have an increase in the level of MDA. In other words, plasma MDA level can be used as biomarker for oxidative stress. [33]

In a study conducted by Yapar et al, liver toxicity induced with paracetamol in mice and hepatoprotective effects of L-Carnitine were investigated, and in this study, MDA values in the blood samples taken 24 hours after paracetamol application decreased in the groups given L-Carnitine while increased paracetamol toxicity group [34]. In another study conducted by Hsu et al., It was found that MDA increased with paracetamol toxicity compared to the healthy group and this increase improved with treatment [35]. In their study, Karakus et al. noted a decrease in liver lipid peroxidation when agomelatine was used. [36]

We also showed that MDA levels increased significantly in the paracetamol group after administration of agomelatine. Thus, we showed that agomelatine has a protective effect on paracetamol-induced nephrotoxicity by improving the MDA level, which increases due to oxidative damage due to paracetamol.

GSH is one of the most important molecules involved in cellular defense against chemically reactive toxic compounds or oxidative stress. As a mediator of oxidative stress at sufficiently high doses of paracetamol, NAPQI is known to lead to a decrease in GSH levels and an increase in lipid peroxidation due to this decrease. This toxic metabolite binds to critical cellular proteins, leading to hepatic necrosis. [38]

In the study conducted by Manda et al., blood GSH levels were found to be decreased in the group liver toxicity induced by paracetamol compared to the control group, and these levels were increased with the given β-carotene treatment [39]. In a similar study by Yaper et al.; serum GSH values were found to be decreased in paracetamol-induced toxicity and it was found that these decreased GSH values increased with the given L-Carnitine treatment. [34]

In the study of Karakus et al., GSH levels decreased in the liver with paracetamol administration and it was shown to be increased again with agomelatine administration [36]. In our study, it was determined that kidney GSH value decreased in paracetamol-induced nephrotoxicity. In our study, we found that the decrease in GSH values in kidney tissues of rats with paracetamol toxicity increased statistically significantly in the groups treated with agomelatine. This result supports the positive effects of agomelatine on the antioxidant system in paracetamol-induced nephrotoxicity.

SOD is a family of enzymes that catalyze the dismutation of superoxide in oxygen and hydrogen peroxide. For this reason, it is an important antioxidant defense mechanism in almost all cells exposed to oxygen and decreases in SOD enzyme activities in paracetamol toxicity [40].

In a study by Gao et al., acute organ damage was induced by administering paracetamol to mice and a decrease in SOD activity was observed in the group where paracetamol toxicity was established [40]. In another study by Xin et al., serum SOD activities were found to be reduced in mice with toxicity by paracetamol. [42] In the study of Karakuş et al., it was noted that SOD activity in the rat liver improved and lipid peroxidation decreased when using agomelatine.[36] In our study, we showed that the decrease in SOD enzyme activity in the toxicity group tended to ameliorate significantly with agomelatine administration. This result supports that agomelatine has a positive effect on paracetamol toxicity on antioxidant enzyme system.

The results of this study, which we examined the effects of agomelatine on acute kidney toxicity with paracetamol are promising. NAC is used in current antidotal treatment in paracetamol overdose. Therefore, we compared the effects of agomelatine with NAC treatment. As a result of experimental studies; serum urea and creatinine levels, which are important indicators of kidney damage, showed an improvement similar to NAC group in agomelatine group. The effects of agomelatine on MDA levels, the primary precursor of tissue damage, were observed to be better than NAC treatment. The effects of agomelatine and NAC on the antioxidant system (SOD and GSH) parameters were found close to each other. In all these results; melatonin receptors may have a role in kidney damage due to paracetamol; It has been demonstrated by administering agomelatine, a melatonin analog. Kidney MDA, serum urea and creatinine levels, which increased due to paracetamol application, decreased with agomelatine administration, kidney SOD activity and GSH levels tended to improve significantly.
5. CONCLUSION

In conclusion, we can say that the data obtained in our study contributed to science in terms of showing that agomelatine can be a new antioxidant agent that can be used in paracetamol poisoning if it is supported by further research.

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