• Basic Research •

Potential effects of adenosine triphosphate and melatonin on oxidative and inflammatory optic nerve damage in rats caused by 5-fluorouracil

Ahmet Mehmet Somuncu[1], Busra Parlak Somuncu[2], Ahmet Duhan Ozbay[3], Ibrahim Cicek[4], Bahadir Suleyman[5], Renad Mammadov[5], Seval Bulut[5], Tugba Bal Tastan[6], Taha Abdulkadir Coban[7], Halis Suleyman[5], Aliyev Aydin[8]

[1]Trabzon Kanuni Education and Research Hospital, Health Sciences University, Trabzon 61250, Turkiey
[2]Provincial Health Directorate, Ministry of Health of the Republic of Turkey, Trabzon 61040, Turkiey
[3]Department of Ophthalmology, Erzurum City Hospital, Health Sciences University, Erzurum 25240, Turkiey
[4]Department of Ophthalmology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkiey
[5]Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkiey
[6]Department of Histology and Embryology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkiey
[7]Department of Biochemistry, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Turkey
[8]Department of Pharmacology, Azerbaijan Medical University, Baku, AZ1022, Azerbaijan

Correspondence to: Halis Suleyman. Department of Pharmacology, Faculty of Medicine, Erzincan Binali Yildirim University, Erzincan 24100, Türkiye. halis.suleyman@gmail.com
Received: 2024-05-19 Accepted: 2024-11-19

Abstract

● **AIM:** To investigate the effects of adenosine triphosphate (ATP) and melatonin, which have antioxidant and anti-inflammatory activities, on potential 5-fluorouracil (5-FU)-induced optic nerve damage in rats.

● **METHODS:** Twenty-four rats were categorized into four groups of six rats: healthy (HG), 5-FU (FUG), ATP+5-FU (AFU), and melatonin+5-FU (MFU). ATP (4 mg/kg) and melatonin (10 mg/kg) were administered intraperitoneally and orally, respectively. One hour after ATP and melatonin administration, rats in the AFU, MFU, and FUG were intraperitoneally injected with 5-FU (100 mg/kg). ATP and melatonin were administered once daily for 10d. 5-FU was administered at a single dose on days 1, 3, and 5 of the experiment. After 10d, the rats were euthanized and optic nerve tissues were extracted. Optic nerve tissues were biochemically and histopathologically examined.

● **RESULTS:** ATP and melatonin treatments inhibited the increase in malondialdehyde (MDA) and interleukin-6 (IL-6) levels, which were elevated in the FUG. The treatments also prevented the decrease in total glutathione (tGSH) levels and the superoxide dismutase (SOD) and catalase (CAT) activities (P<0.001). This inhibition was higher in the ATP group than in the melatonin group (P<0.001). ATP prevented histopathological damage better than melatonin (P<0.05).

● **CONCLUSION:** ATP and melatonin have the potential to be used in alleviating 5-FU-induced optic nerve damage. In addition, ATP treatment shows better protective effects than melatonin.

● **KEYWORDS:** adenosine triphosphate; melatonin; 5-fluorouracil; optic nerve damage; antioxidant; anti-inflammatory

DOI:10.18240/ijo.2025.02.04

Citation: Somuncu AM, Parlak Somuncu B, Ozbay AD, Cicek I, Suleyman B, Mammadov R, Bulut S, Bal Tastan T, Coban TA, Suleyman H, Aydin A. Potential effects of adenosine triphosphate and melatonin on oxidative and inflammatory optic nerve damage in rats caused by 5-fluorouracil. *Int J Ophthalmol* 2025;18(2):222-228

INTRODUCTION

5-fluorouracil (5-FU) is an antimetabolite drug that inhibits deoxyribonucleic acid and protein synthesis *via* thymidylate synthase inhibition and ribonucleic acid integration[1-2]. 5-FU is an important anticancer drug among the broad-spectrum chemotherapeutics[3]. 5-FU, which affects the "S" phase of cancer cells, is used in the treatment of breast, pancreatic, and colorectal cancers[4-5]. However, its toxicity to normal tissues is a primary obstacle to successful cancer chemotherapy. Further, its serious side effects lead to the discontinuation of 5-FU treatment[3]. The use of 5-FU has been associated with multiple organ dysfunction[6]. For example,

Raina *et al*[7] reported a case involving 5-FU-associated optic neuropathy. 5-FU-associated optic neuropathy was documented in another patient with dihydropyrimidine dehydrogenase deficiency[8]. However, exacerbation of peripheral neuropathy has been reported even in individuals with normal dihydropyrimidine dehydrogenase levels[9]. The toxicity of 5-FU on the optic nerve has been attributed to oxidative stress and an increase in pro-inflammatory cytokines[10]. 5-FU also decreases intracellular adenosine triphosphate (ATP) levels, suggesting that a reduction in intracellular ATP levels may be a key factor underlying 5-FU-induced oxidative damage[11]. This is supported with studies reporting that ATP participates in the synthesis of reactive oxygen species' (ROS) scavenging antioxidants[12]. Furthermore, ATP is used as an energy source in the production of low molecular weight antioxidants[13]. Ozer *et al*[14] showed that ATP protects ovaries from oxidative and inflammatory damage associated with 5-FU by suppressing oxidant and proinflammatory cytokine production.

Melatonin (N-acetyl-5-methoxytryptamine) is a molecule found in almost all living organisms. It is primarily produced by the pineal gland and released directly into the bloodstream, where it serves as a hormone[15]. It is an important regulator of physiological processes with antioxidant and anti-inflammatory activities[16]. Melatonin has been shown to increase intracellular ATP levels[17]. Several studies have reported that melatonin protects mitochondria against oxidative damage by increasing ATP production[18]. The information obtained from the literature suggest that ATP and melatonin may protect optic nerves from potential oxidative and inflammatory injury caused by 5-FU. However, there are no studies investigating the effects of ATP and melatonin against 5-FU-induced optic nerve damage. Therefore, we aimed to investigate the effects of ATP and melatonin biochemically and histopathologically against potential optic nerve injury caused by 5-FU in rats and compare their effects.

MATERIALS AND METHODS

Ethical Approval Experimental applications were carried out in the laboratories of Erzincan Binali Yıldırım University Experimental Animals Application and Research Centre. The experimental procedures were performed after the approval of the Local Ethics Committee for Animal Experiments (date: 29.09.2023, approval number: 32).

Animals Twenty-four male albino Wistar rats (weight: 281±6 g; age: 5–6mo) were used in the study. Rats were acquired from Experimental Animal Research and Application Center of Erzincan Binali Yıldırım University. The rats were housed in a laboratory with a room temperature of 22°C±2°C, an automatic alternating 12-h dark-light cycle, and fed *ad libitum*.

Chemicals Thiopental sodium (1-g vial) was acquired from IE Ulagay (Istanbul, Turkey), ATP was from Zdorove Narodu (Ukraine), melatonin (3-mg tablet) was from Przedsiebiorstwo Farmaceutczne LekAm (Zakroczym, Poland), and 5-FU (1000 mg/20 mL intravenous solution) was from Hospital of the Ministry of Health (Erzincan, Turkey).

Experimental Groups Four groups of six animals were formed (n=6/each group): healthy (HG), 5-FU (FUG), ATP+5-FU (AFU), and melatonin+5-FU (MFU).

Experimental Procedure The experimental procedure was started with ATP and melatonin treatments. ATP (4 mg/kg)[19] was intraperitoneally (*i.p.*) administered to the AFU group. Melatonin (10 mg/kg)[20] was given using oral gavage to the the MFU group. At this stage, the same volume of *i.p*. 0.9% sodium chloride was given to HG and FUG groups. One hour after the administration of ATP, melatonin, and 0.9% sodium chloride, rats in the AFU, MFU, and FUG groups were *i.p*. injected with 5-FU (100 mg/kg)[10]. ATP and melatonin treatments were continued once daily for a period of 10d. 5-FU treatment was administered as a single dose on days 1, 3, and 5 of the experiment. After 10d, rats were euthanized by *i.p.* administration of thiopental sodium (50 mg/kg). Optic nerve tissues were extracted. Tissues were analysed for malondialdehyde (MDA), total glutathione (tGSH), superoxide dismutase (SOD), catalase (CAT), protein and interleukin-6 (IL-6) and examined histopathologically.

Biochemical Analyses

MDA, tGSH, SOD, CAT, and IL-6 analysis The tissues were powdered by rapid grinding in liquid nitrogen and homogenized. Clear filtrate was used for analyses. Samples were stored at -80°C until analysis. For the analyses, MDA, tGSH and SOD in optic nerve tissues, enzyme-linked immunosorbent assay kits designed for rats were used (product numbers 706002, 703002, and 10009055, Cayman Chemical Company, respectively). CAT activity were determined according to the methods of Goth[21]. Enzyme-linked immunosorbent assay kit (Eastbiopharm Co Ltd., China) was used to determine IL-6 levels.

Histopathological Procedures Haematoxylin-eosin method: for tissue stabilisation, tissues were stored in 10% formalin solution and embedded in paraffin after routine procedures. The 5 μm sections obtained from the blocks were stained with Haematoxylin-Eosin and examined under light microscope. Histopathological changes in optic nerve tissue were defined as destruction, polymorphonuclear cell infiltration, increased thickness, increased astrocyte cell population, and presence of edema/vacuolization. The samples were semiquantitatively evaluated. Histopathological findings were scored as absent (0), mild (1), moderate (2), and severe (3).

Statistical Analysis Statistical analyses were performed using the statistical program Statistical Package for the Social Sciences for Windows, version 22.0. Biochemical data

Table 1 Results of analysis of MDA, tGSH, SOD, CAT, and IL-6 data measured from optic nerve tissues mean±SD

Parameters	HG	FUG	AFU	MFU	$F_{(3, 20)}/P$
MDA (nmol/mg protein)	4.32±0.14[a]	8.80±0.12	4.88±029[a,b,c]	6.73±0.70[a]	162.747/<0.001
tGSH (nmol/mg protein)	9.28±0.15[a]	4.45±0.24	8.85±0.38[a,c]	6.54±0.19[a]	466.428/<0.001
SOD (U/mg protein)	7.82±0.12[a]	3.67±0.11	7.45±0.67[a,c]	5.37±0.12[a]	184.450/<0.001
CAT (U/mg protein)	7.17±0.09[a]	3.18±0.12	6.96±0.13[a,c]	4.85±0.11[a]	1697.006/<0.001
IL-6 (ng/L)	2.79±0.15[a]	6.35±0.12	3.03±014[a,c]	4.80±0.09[a]	1082.153/<0.001

[a]*P*<0.001 *vs* FUG; [b]*P*<0.001 *vs* MFU; [c]*P*>0.05 *vs* HG. MDA: Malondialdehyde, tGSH: Total glutathione; SOD: Superoxide dismutase; CAT: Catalase; IL-6: Interleukin 6; HG: Healthy group; FUG: 5-fluorouracil group; AFU: ATP+5-fluorouracil group; MFU: Melatonin+5-fluorouracil group. Statistical analysis was performed by one way ANOVA-Tukey or Games-Howell tests.

Table 2 Histopathological scoring analysis results of optic nerve tissues median (quartile 1–quartile 3)

Parameters	HG	FUG	AFU	MFU	*H*/*P*
Destruction	0 (0–0)[a]	3 (2–3)	0 (0–1)[a,b,c]	1 (1–2)[a]	103.896/<0.001
PMNL presence	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)	0.000/1.000
Increase in connective tissue thickness	0 (0–0)[a]	2 (2–3)	0 (0–1)[a,b,c]	1.5 (1–2)[a]	113.453/<0.001
Increase in astrocyte cell	0 (0–0)[a]	2 (2–2)	0 (0–1)[a,b,c]	1 (1–2)[a]	97.596/<0.001
Edema/vacuolization	0 (0–0)[a]	3 (2–3)	1 (0–1)[a,b]	2 (1–2)[a]	107.094/<0.001

Histopatological grading; 0: absent, 1: mild damage, 2: moderate damage, 3: severe damage. [a]*P*<0.001 *vs* FUG; [b]*P*<0.001 *vs* MFU; [c]*P*>0.05 *vs* HG. PMNL: Polymorphonuclear cell infiltration; HG: Healthy group; FUG: 5-fluorouracil group; AFU: ATP+5-fluorouracil group; MFU: Melatonin+5-fluorouracil group. Statistical analysis was performed by Kruskal Wallis test-Dunn's test.

were expressed as mean value±standard deviation. Shapiro-Wilk test confirmed that the data conformed to normal distribution, and one-way ANOVA test was used for analyses. Subsequently, Tukey's honestly significant differenceip or Games-Howell post-hoc tests were performed based on the homogeneity of variances. For the analysis of semiquantitative histopathological scoring data, the Kruskal-Wallis test was used, followed by the post-hoc Dunn test. Data were presented as median (interquartile range). $P<0.05$ was considered statistically significant.

RESULTS

Biochemical Findings

MDA levels in optic nerve tissues MDA levels were increased in FU group compared with that of healthy group ($P<0.001$). Both ATP and melatonin suppressed the 5-FU-induced increase in MDA ($P<0.001$). The inhibition was more pronounced in AFU group compared with that in the MFU ($P<0.001$). MDA levels in rats in AFU group were similar to those in healthy group ($P=0.094$; Table 1).

tGSH levels in optic nerve tissues 5-FU treatment decreased tGSH levels compared to healthy rats ($P<0.001$). tGSH levels were higher in rats receiving ATP and melatonin compared to the 5-FU group ($P>0.001$). ATP maintained tGSH levels better than melatonin ($P<0.001$; Table 1).

SOD and CAT activities levels in optic nerve tissues Compared to healthy rats, 5-FU treatment decreased SOD and CAT activities in optic nerve samples ($P<0.001$). This decrease was suppressed by ATP and melatonin ($P<0.001$). ATP had a stronger protective effect on SOD and CAT activities compared with that of melatonin ($P<0.001$). In terms of tissue SOD activities, the differences between the AFU and healthy groups were insignificant ($P=0.290$; Table 1).

IL-6 levels in optic nerve tissues IL-6 levels were increased in the 5-FU group compared with those in the healthy group ($P<0.001$). In contrast, IL-6 levels were lower in the AFU and MFU groups compared with the 5-FU group ($P<0.001$). The use of ATP better inhibited the increase in IL-6 levels compared with melatonin use ($P<0.001$; Table 1).

Histopathological Findings In the tissue samples of healthy group, the connective tissue thickness surrounding the optic nerve was normal. Axon areas were stained as slightly eosinophilic, and glial cell nuclei observed between junctions and axons were stained as basophilic. Astrocytes and their extensions appeared normal. Normal blood vessels were occasionally present in the optic nerve trabeculae (Figure 1A; Table 2).

Optic nerve tissue samples of the FU group were generally vacuolized and edematous. There was a noticeable increase in the thickness of the connective tissue surrounding the optic nerve. In the tissue samples, there was an increased number of hypertrophic and degenerated astrocytes. Findings of dilatation and congestion were observed in the blood vessels (Figure 1B; Table 2).

In the optic nerve tissue images of the AFU group, the thickness of the connective tissue surrounding the optic nerve was similar to that of the healthy group. There was a mild presence of vacuolization and edema throughout the tissue than that in the FU group. The overall morphology of astrocytes

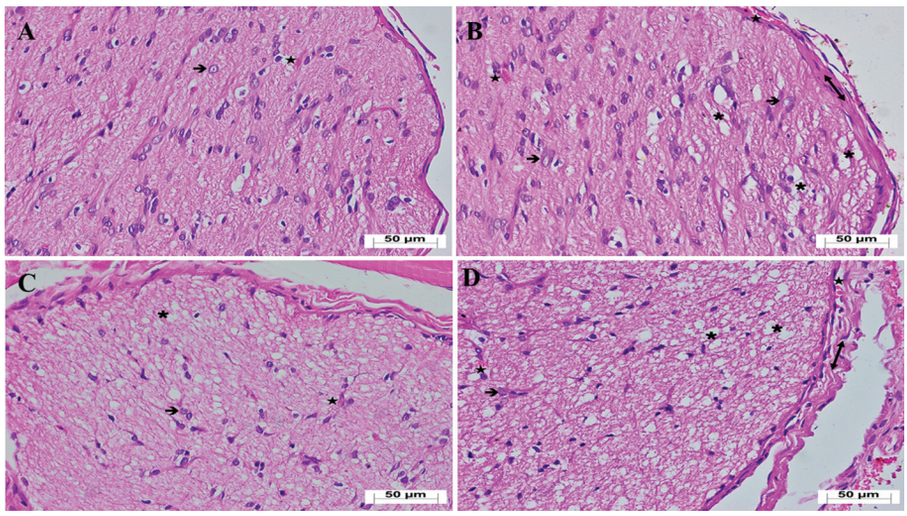

Figure 1 Histopathological appearances of optic nerve tissues obtained from experimental groups A: Optic nerve tissue in the HG; ➔: Astrocyte, ★: Blood capillary, x400. B: Optic nerve tissue in the FUG; ➔: Hypertrophied and degenerated astrocyte, ★: Blood capillary, ✱: Vacuolization, two headed arrow: Increase of thickness x400. C: Optic nerve tissue in the AFU; ➔: Astrocyte, ★: Blood capillary, ✱: Vacuolization, x400. D: Optic nerve tissue in the MFU; ➔: Hypertrophied and degenerated astrocyte, ★: Blood capillary, ✱: Vacuolization, two headed arrow: Increase of thickness. Hematoxylin-eosin staining, x400. HG: Healthy group; FUG: 5-fluorouracil group; AFU: ATP+5-fluorouracil group; MFU: Melatonin+5-fluorouracil group.

was similar to that of the healthy group. Blood vessels had a near normal appearance (Figure 1C; Table 2).

In the optic nerve tissue samples of the MFU group, the thickness of the surrounding connective tissue remained at a moderate level compared with that of the FU group. Hypertrophic and degenerated astrocytes were still present, although they were less common compared with those of the FU group. The congestion in the blood vessels and the findings of vacuolization and edema of varying sizes throughout the tissue were moderate (Figure 1D; Table 2).

DISCUSSION

In this study, the protective effects of ATP and melatonin treatments against potential damage to the optic nerve tissue caused by 5-FU were investigated through biochemical analysis and histopathological evaluation. 5-FU is reported to increase the amount of ROS and interferes with the cellular antioxidant defense system[22]. ROS are highly reactive chemicals formed from molecular oxygen. At appropriate concentrations, ROS regulate signalling pathways and control specific physiological responses, while excess causes tissue stress[23]. This condition, also defined as the disruption of cellular redox balance, leads to lipid peroxidation and the generation of toxic products such as MDA[24]. Our biochemical findings showed that 5-FU treatment increased MDA levels in the optic nerve, similar to the results of Cicek *et al*[10] 5-FU is known to disrupt mitochondrial energy metabolism through its metabolites and inhibit ATP production[25]. Decreased ATP levels are reported to increase lipid peroxidation[26]. In the present study, ATP administration was found to inhibit the 5-FU-induced increase in MDA levels in optic nerve tissues of rats. Icel *et al*[19] also showed that ATP treatment suppressed the increase in MDA in methanol-induced oxidative optic nerve damage. Melatonin, tested in the present study, also suppressed the increase in MDA levels. However, this effect was not as pronounced as that of ATP. Melatonin has been described as an antioxidant and anti-inflammatory molecule that can attenuate cell damage due to oxidative stress and inflammation[27]. Melatonin has also been used in an experimental optic neuritis model and reported to inhibit the increase in tissue MDA levels[27].

Living organisms resist oxidative damage through enzymatic and nonenzymatic antioxidants[28]. Therefore, tGSH levels were also measured in optic nerve tissues. GSH directly neutralizes oxidizing agents or reduces hydrogen peroxide to water in the presence of glutathione peroxidase[28]. GSH depletion is considered an important indicator of cell death processes[28]. In the present study, tGSH levels were lower in the 5-FU group rats compared with those in the healthy group. Previous experimental studies also reported that 5-FU administration decreased tissue GSH levels[10,14,29]. In contrast to the decreased tGSH levels in the 5-FU group rats, tGSH levels were significantly preserved in the AFU group rats. GSH is synthesized by two enzymatic reactions, both of which require ATP[28]. ATP treatment has previously been tested in the oxidative damage of optic nerve and ovarian tissues and was found to prevent the decrease in GSH levels[19,30]. Furthermore, our results showed that melatonin treatment also inhibited 5-FU-induced tGSH depletion. To the best of our knowledge, there are no studies on the effect of melatonin on tGSH levels in optic neuropathy. However, melatonin has been used in

oxidative sciatic nerve injury and found to help maintain GSH levels[31].

In the present study, SOD and CAT activities in optic nerve tissues were also measured. SOD is the first line of defense against ROS and converts the highly reactive superoxide radical into the less toxic hydrogen peroxide[32-33]. CAT neutralizes hydrogen peroxide by decomposing it into molecular oxygen and water[32]. Our experimental results showed that 5-FU decreased both SOD and CAT activities, similar to the findings of Cicek *et al*[10]. Furthermore, 5-FU was shown to decrease SOD and CAT activities in liver tissue, and the authors stated that 5-FU impaired antioxidant capacity[34]. In the present study, ATP co-administered with 5-FU significantly preserved tissue SOD and CAT activities. ATP treatment has been reported to prevent the decrease in SOD and CAT activities in amiodarone-induced optic neuropathy[30]. Our biochemical results indicated that melatonin treatment, while not as effective as ATP, suppressed the decrease in SOD and CAT activities. Melatonin is reported to prevent the decrease in SOD and CAT activities due to ROS in many tissues in addition to its direct ROS scavenging property[35]. There are numerous studies focused on the antioxidant effects of melatonin[35]. In contrast, studies on the antioxidant effects of ATP treatment are very limited. To the best of our knowledge, no studies are comparing the antioxidative effects of ATP and melatonin. However, our results revealed that although melatonin is known to improve ATP levels, it exhibited a weaker antioxidant effect than exogenously administered ATP[31].

IL-6 levels in the optic nerve tissues were also evaluated in the present study. Cicek *et al*[10] attributed the pathogenesis of 5-FU cytotoxicity to increased inflammatory cytokines. Similar to the findings of Cicek *et al*[10], IL-6 levels were high in the optic nerve tissues of the 5-FU group. 5-FU-induced optic neuropathy has been attributed to ischemic causes due to vasospasm[7]. The fact that optic nerve ischaemia activates inflammatory processes as well as a decrease in ATP production may be one of the mechanisms underlying the increase in IL-6 by 5-FU[36-37]. As evident from our biochemical findings, the increase in IL-6 levels was significantly inhibited by ATP treatment. Ozer *et al*[14] also showed that ATP inhibited the increase in IL-6 levels in 5-FU-induced oxidative ovarian damage. In the present study, IL-6 levels in melatonin-treated rats were lower than those in the 5-FU group rats but higher than that in the ATP group. To the best of our knowledge, there are no studies on the effect of melatonin on tissue IL-6 levels in optic neuropathy. However, melatonin treatment has been reported to exhibit anti-inflammatory activity in an experimental optic neuritis model and has been shown to decrease TNF-α levels[27].

The present study also included histopathological examination of the tissues. Our results revealed that 5-FU treatment caused disruptions in the histological structures of the optic nerves. A recent study showed that 5-FU caused degenerated astrocytes, severe edema, and vacuolization in optic nerve tissues[10]. In the present study, histopathological evaluation of the sections in the AFU group revealed that vasculature, connective tissue, and astrocytes were normal with only mild vacuolization and edema. Similarly, Bayrakçeken *et al*[30] tested ATP in optic neuropathy in rats and found that the treatment reduced degeneration and vacuolisation, suppressed the increase in connective tissue and astrocyte increase. In the present study, melatonin treatment was also successful in preventing 5-FU-induced damage, although not as effective as ATP. In the optic nerve tissue samples of the melatonin group, thickening of the connective tissue and changes in astrocytes and vascular structures were reduced but still persisted at a moderate level. A previous study showed that melatonin treatment reduced kidney damage in the renal tissues of mice with 5-FU-induced nephrotoxicity[38].

Limitations We believe that investigating the combined effect of melatonin and ATP on 5-FU-induced optic damage and measuring anti-inflammatory cytokine levels can be useful in elucidating the pathogenesis and treatment of optic nerve damage.

In conclusion, we observed that in the optic nerves of the rats in the 5-FU group, the balance between oxidants and antioxidants was disrupted in favor of oxidants, and an increase in cytokines responsible for inflammatory processes was detected. Biochemical data and histopathological examination showed that 5-FU caused oxidative and inflammatory damage in optic nerve tissue. According to our results, both ATP and melatonin have the potential to be used in alleviating 5-FU-induced optic nerve damage. However, ATP treatment showed a better protective effect than melatonin. To date, there are few studies on the use of exogenous ATP. The use of ATP can be a new treatment strategy in many diseases where oxidative and inflammatory processes are involved in the pathogenesis. Therefore, it is important to conduct future studies to elucidate the mechanisms underlying the protective effect of ATP.

ACKNOWLEDGEMENTS

Conflicts of Interest: Somuncu AM, None; **Parlak Somuncu B,** None; **Ozbay AD,** None; **Cicek I,** None; **Suleyman B,** None; **Mammadov R,** None; **Bulut S,** None; **Bal Tastan T,** None; **Coban TA,** None; **Suleyman H,** None; **Aydin A,** None.

REFERENCES

1 dos Santos AM, Junior AGT, Carvalho SG, *et al.* An updated review on properties, nanodelivery systems, and analytical methods for the determination of 5-fluorouracil in pharmaceutical and biological samples. *Curr Pharm Des* 2022;28(18):1501-1512.

2 Longley DB, Harkin DP, Johnston PG. 5-fluorouracil: mechanisms of action and clinical strategies. *Nat Rev Cancer* 2003;3(5):330-338.

3 Ciaffaglione V, Modica MN, Pittalà V, *et al.* Mutual prodrugs of 5-fluorouracil: from a classic chemotherapeutic agent to novel potential anticancer drugs. *ChemMedChem* 2021;16(23):3496-3512.

4 Zhang N, Yin Y, Xu SJ, *et al.* 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules* 2008;13(8):1551-1569.

5 Valencia-Lazcano AA, Hassan D, Pourmadadi M, *et al.* 5-Fluorouracil nano-delivery systems as a cutting-edge for cancer therapy. *Eur J Med Chem* 2023;246:114995.

6 Lazar A, Mau-Holzmann UA, Kolb H, *et al*. Multiple organ failure due to 5-fluorouracil chemotherapy in a patient with a rare dihydropyrimidine dehydrogenase gene variant. *Onkologie* 2004;27(6):559-562.

7 Raina AJ, Gilbar PJ, Grewal GD, *et al.* Optic neuritis induced by 5-fluorouracil chemotherapy: case report and review of the literature. *J Oncol Pharm Pract* 2020;26(2):511-516.

8 Delval L, Klastersky J. Optic neuropathy in cancer patients. Report of a case possibly related to 5 fluorouracil toxicity and review of the literature. *J Neurooncol* 2002;60(2):165-169.

9 Saif MW, Hashmi S, Mattison L, *et al*. Peripheral neuropathy exacerbation associated with topical 5-fluorouracil. *Anticancer Drugs* 2006;17(9):1095-1098.

10 Cicek I, Altuner D, Suleyman Z, *et al.* The effect of carvacrol on 5-fluorouracil-induced optic neuropathy in rats. histopathological and biochemical analysis. *Lat Am J Pharm* 2023;42(11):2223-2230.

11 Zhang DX, Ma JT. Mitochondrial dynamics in rat heart induced by 5-fluorouracil. *Med Sci Monit* 2018;24:6666-6672.

12 Saquet AA, Streif J, Bangerth F. Changes in ATP, ADP and pyridine nucleotide levels related to the incidence of physiological disorders in 'Conference' pears and 'Jonagold' apples during controlled atmosphere storage. *J Hortic Sci Biotechnol* 2000;75(2):243-249.

13 Yi C, Jiang YM, Shi J, *et al.* ATP-regulation of antioxidant properties and phenolics in litchi fruit during browning and pathogen infection process. *Food Chem* 2010;118(1):42-47.

14 Ozer M, Ince S, Altuner D, *et al.* Protective effect of adenosine triphosphate against 5-fluorouracil-induced oxidative ovarian damage *in vivo*. *Asian Pac J Cancer Prev* 2023;24(3):1007-1013.

15 Amaral FGD, Cipolla-Neto J. A brief review about melatonin, a pineal hormone. *Arch Endocrinol Metab* 2018;62(4):472-479.

16 Chitimus DM, Popescu MR, Voiculescu SE, *et al.* Melatonin's impact on antioxidative and anti-inflammatory reprogramming in homeostasis and disease. *Biomolecules* 2020;10(9):1211.

17 Teng ZY, Zheng WW, Jiang SF, *et al.* Role of melatonin in promoting plant growth by regulating carbon assimilation and ATP accumulation. *Plant Sci* 2022;319:111276.

18 Wesselink E, Koekkoek WAC, Grefte S, *et al.* Feeding mitochondria: potential role of nutritional components to improve critical illness convalescence. *Clin Nutr* 2019;38(3):982-995.

19 Icel E, Suleyman H, Yazici GN, *et al.* Effects of adenosine triphosphate on methanol-induced experimental optic nerve damage in rats: biochemical and histopathological evaluation. *Cutan Ocul Toxicol* 2020;39(3):244-248.

20 Verma AK, Singh S, Garg G, *et al.* Melatonin exerts neuroprotection in a chronodisrupted rat model through reduction in oxidative stress and modulation of autophagy. *Chronobiol Int* 2022;39(1):45-56.

21 Góth L. A simple method for determination of serum catalase activity and revision of reference range. *Clin Chim Acta* 1991;196(2-3):143-151.

22 Domanskyi A, Parlato R. Oxidative stress in neurodegenerative diseases. *Antioxidants* (*Basel*) 2022;11(3):504.

23 Jing RH, Hu CH, Qi TT, *et al.* Role of reactive oxygen species in epithelial-mesenchymal transition and apoptosis of human lens epithelial cells. *Int J Ophthalmol* 2023;16(12):1935-1941.

24 Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. *Eur J Med Chem* 2015;97:55-74.

25 Lischke J, Lang C, Sawodny O, *et al*. Impairment of energy metabolism in cardiomyocytes caused by 5-FU catabolites can be compensated by administration of amino acids. *2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)*. August 25–29, 2015, Milan, Italy. IEEE, 2015:5363-5366.

26 Song SB, Hwang ES. A rise in ATP, ROS, and mitochondrial content upon glucose withdrawal correlates with a dysregulated mitochondria turnover mediated by the activation of the protein deacetylase SIRT1. *Cells* 2018;8(1):11.

27 Aranda ML, González Fleitas MF, De Laurentiis A, *et al*. Neuroprotective effect of melatonin in experimental optic neuritis in rats. *J Pineal Res* 2016;60(3):360-372.

28 Tan MY, Yin YF, Ma X, *et al.* Glutathione system enhancement for cardiac protection: pharmacological options against oxidative stress and ferroptosis. *Cell Death Dis* 2023;14(2):131.

29 Gelen V, Şengül E, Yıldırım S, *et al*. The protective effects of hesperidin and curcumin on 5-fluorouracil-induced nephrotoxicity in mice. *Environ Sci Pollut Res Int* 2021;28(34):47046-47055.

30 Bayrakçeken K, Ucgul RK, Coban T, *et al*. Effect of adenosine triphosphate on amiodarone-induced optic neuropathy in rats: biochemical and histopathological evaluation. *Cutan Ocul Toxicol* 2023;42(3):162-167.

31 Areti A, Komirishetty P, Akuthota M, *et al.* Melatonin prevents mitochondrial dysfunction and promotes neuroprotection by inducing autophagy during oxaliplatin-evoked peripheral neuropathy. *J Pineal Res* 2017;62(3).

32 He L, He T, Farrar S, *et al.* Antioxidants maintain cellular redox homeostasis by elimination of reactive oxygen species. *Cell Physiol Biochem* 2017;44(2):532-553.

33 Chen Y, Shen J, Zhang X, *et al*. Protective effects of ferulic acid against ionizing radiation-induced oxidative damage in rat lens through activating Nrf2 signal pathway. *Int J Ophthalmol* 2023;16(5):687-693.

34 Zeng D, Wang YP, Chen Y, *et al.* Angelica polysaccharide antagonizes

5-FU-induced oxidative stress injury to reduce apoptosis in the liver through Nrf2 pathway. *Front Oncol* 2021;11:720620.

35 Rodriguez C, Mayo JC, Sainz RM, *et al.* Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 2004;36(1):1-9.

36 Vilares-Morgado R, Nunes HMM, dos Reis RS, *et al.* Management of ocular arterial ischemic diseases: a review. *Graefes Arch Clin Exp Ophthalmol* 2023;261(1):1-22.

37 Zhang ZQ, Xie Z, Chen SY, *et al.* Mitochondrial dysfunction in glaucomatous degeneration. *Int J Ophthalmol*. 2023;16(5):811-823.

38 Elbanan ME, Amer ME, El-Missiry MA, *et al.* Melatonin protected against kidney impairment induced by 5-fluorouracil in mice. *J Exp Zool A Ecol Integr Physiol* 2023;339(8):777-787.