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Neuroprotective effects of agmatine in antineoplastic drugs induced neurotoxicity: In vitro study

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ABSTRACT

Aims: The effects of agmatine, an endogenous substance known to have a neuroprotective effect against neurotoxicity has been investigated.
Material and methods: The primary neuron culture obtained from neonatal rats was exposed to toxicity with paclitaxel and cisplatin and the effect of agmatine on both acute (1 h) and chronic (24 h) exposure was demonstrated by biochemical and molecular analyses. It was demonstrated that the effect of agmatine before and after agmatine was induced by neurotoxicity before agmatine and the effect of agmatine on the formed and occuring toxicities. In addition to the results of cell viability assay, total oxidant capacity and total antioxidant capacity, we have found the opportunity to elaborate on our molecular mechanisms by elaborating our findings with apoptotic and inflammation markers such as caspase 3, kaspase 9 and TNF alpha.
Key findings: The results of our study revealed the effect profile of a protective molecule against pathological neural deaths due to neurodegeneration not only in neurotoxicity due to anticancer drugs.
Significance: In this context, we tried to reverse neurotoxicity due to anticancer drugs by using agmatine the duration (1 and 24 h) and dosage (10–5 M and 10–6 M) determined.

1. Introduction

Cancer is the most important cause of death after death due to cardiovascular diseases today. It has a high morbidity and mortality rate and is still scientifically relevant as it cannot yet be fully treated [1]. It is estimated that cancer-related morbidity and mortality will increase gradually due to the increase in the elderly population in the coming years [2,3]. Antineoplastic agents used in the treatment of cancer are important due to the increase in the incidence of cancer and the increase in people living with cancer. Chemotherapy drugs can cause a number of uncomfortable side effects in the individual in relation to their effects [4,5].

Cisplatin and paclitaxel are the major antineoplastic agents in treatment regimes that frequently cause acute and delayed central nervous system toxicity. Neurotoxicity caused by antineoplastic agents such as cisplatin and paclitaxel has excitotoxic properties that develop due to glutamate. Neuronal cell death is a cellular event that can be triggered in intracellular and extracellular pathological conditions, such as synaptogenesis, histogenesis, and tissue homoeostasis. In many acute and chronic brain damage, such as hypoxia, ischemia, trauma, epilepsy, neurodegeneration and neurotoxicity, toxic stimulation results in neuronal death. It is known that neurotoxicity caused by antineoplastic agents is caused by dragging the cell to apoptosis. Many studies are being carried out today to prevent this neurotoxicity. However, such problems still persist in patients after cancer chemotherapy and they negatively affect the patient's life after treatment. For this reason, both clinical and preclinical studies are continuing for the prevention of neurotoxicity, especially developing due to cancer chemotherapy.

Agmatine, an endogenous substance known to modulate many functions in the nervous system, is an organic cation. Agmatin is synthesized from the L-arginine and decarboxylase of arginine, which is its specific enzyme in the nervous cell in the central nervous system. It is released from the presynaptic end as a result of Ca^{+2} dependent depolarization. With agmatinase enzyme, putresine is converted into polyamines such as spermidine, spermine. Agmatin has a wide spectrum of pharmacological effects by binding on various receptors and taking part in important functions in periphery and center [6,7]. The neuroprotective effects of agmatine were determined in 1995. Several studies have shown that there are cytoprotective effects, including neuroprotective, cardioprotective and nephroprotective effects. It has

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been shown to have neuroprotective effects in cases such as stroke, central nervous system traumas and neuropathic pain. These neuroprotective effects are, especially the modulation of neurotransmitter receptors such as N-methyl D-aspartate, modulation of ion channels such as calcium and inhibition of nitric oxide synthesis. For this reason, agmatin has been shown to be promising both as a preventive (neuroprotective) agent and as a neurotransmitter therapy after cerebrovascular events [8]. This study focuses on agmatine, which is known to have an endogenous neuroprotective effect. In this context, we tried to reverse neurotoxicity due to anticancer drugs by using agmatine for the duration and dosage determined according to the dose-duration-effect profile study results previously performed on agmatine.

2. Materials and methods

2.1. Animal supply and ethics committee

This study was conducted with permission from the Local Ethics Committee of Kafkas University Animal Experiments dated 25.01.2016 numbered 2016–016. A total of 10 newborn Spraque Dawley rats obtained from the experimental animal laboratory were used.

2.2. Primary neuron culture

The newborn Sprague Dawley rats were rapidly decapitated and the cortex neurons were taken and stored in the prepared solution. After adding 1:1 tyripsin and waiting for 30 min in the incubator, centrifuge was done three times, supernatant was discarded each time and new medium added. In a separate tube, a neurobasal medium prepared with 1000:1 penicillin 50:1 B27 and 10:1 FBS (fetal bovine serum) was prepared. Cells were added to the prepared medium. 150 µl of medium were added to each well of 96 well plates. 3 cc of medium was added to the 6-well plates. The cells were kept in the incubator for 10 days to adhere to the floor of the chamber and to cover the floor and to grow.

2.3. Chemicals ans Drug Application

Paclitaxel (Taxol® 6 mg/mL vial) was purchased from Bristol-Myers Squibb (New York, NY, USA) and Cisplatin (10 mg/mL vial) was purchased from Pharmachemie BV (Harlem, Holland). Agmatine (CAS Number 0002482000), Dulbecco's Modified Eagle's Medium (DMEM), Hanks' Balanced Salt solution (HBSS) and DNase type 1 were purchased from Sigma Aldrich® (Steinheim, Germany). Neurobasal medium (NBM) and fetal calf serum (FCS) were purchased from Gibco-Life Technologies (Australia Pty Ltd, Mulgrave, VIC, Australia) Cell cultures prepared for the study were exposed to agmatine 10^{-5} M and 10^{-6} M for 1 h prior to forming toxicity with 10^{-7} and 10^{-8} M paclitaxel and 50 and 100 mM cisplatin, and 1 h and 24 h after. In our project, agmatine was exposed for 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h and 24 h. This study was designed with the durations (1 h and 24 h) where the protective effect was observed.

1. Agmatine exposure for 1 h before toxicity (4 × 96 well plates)
2. Agmatine exposure for 1 h after toxicity (4 × 96 well plates)
3. 24 h agmatine exposure after toxicity (4 × 96 well plates)

to a total of 10 wells per group. A total of 1152 cultures were obtained.

2.4. Vitality tests

The number of dead/live cells was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) proliferation kit (Cayman Chemical, Ann Arbor, Mi, USA) described as previously [9,10].

2.5. Total antioxidant capacity (TAC) and Total oxidant capacity (TOC) analysis

In paclitaxel and cisplatin toxicity, commercial kit was used to determine the TAC and TOC levels of neuroprotective endogenous molecule agmatine on primary neuron neuron culture cells (Rel Assay Diagnostics, Gaziantep, Turkey). The kit application is calibrated with a stable antioxidant, vitamin E analogy and called Trolox equivalent [11,12]. A commercial TOC kit was used to determine the TOC levels of neuroprotective endogenous molecule agmatine on primary neuronal culture cells in paclitaxel and cisplatin toxicity (Rel Assay Diagnostics, Gaziantep, Turkey).

2.6. Molecular analysis

For RT PCR analysis, 9 groups (Control, Agmatine 10^{-5} M, Agmatine 10^{-6} M, Paclitaxel, Cisplatin, Paclitaxel + Agmatine 10^{-5} M, Paclitaxel + Agmatine 10^{-6} M, Cisplatin + Agmatine 10^{-5} M, Cisplatin + Agmatine 10^{-6} M,) were created to work on the most effective doses according to MTT and TAC/TOC levels.

2.6.1. RNA isolation and RT-PCR measurements

In our study, the expression levels of mRNA, caspase 3, caspase 9 and TNF alpha, were compared between groups. RNA extraction from cells: When the cells were plated on 6-well plates, drug administration procedures were performed and cells were removed by removing the medium from the plates. Tissue LYSER II (Qiagen) cells were homogenized and RNA extraction was performed with Qiacube. Using the RNeasy Mini Kit (Qiagen), the total RNA isolation phases from cell samples using the the Qiaqube RNA isolation device (Qiagen) were continued as recommended by the manufacturer. Total mRNA was measured at 260 nm with nano drop spectrophotometry (EPOCH, Biotek) [13].

2.6.2. Reverse transcriptase reaction and cDNA synthesis

cDNA synthesis from total RNA was performed using Roche cDNA Reverse Transcription Kit enzyme. Each reaction was performed with 10 µl of RNA and the cDNA synthesis was performed with Veriti 96 Well Roch Light Cycler according to the following temperature values. We used nano drop (spectrophotometry based assay) (EPOCH Plate, Biotek) for masuring the amount of cDNA The quantitative determination of real time PCR and mRNA expressions was performed using the Roche Gene Expression Master Mix kit. Amplification and quantification was performed on the Roche Light Cycler. The 100 ng cDNA was pipetted as shown in the table below withRoche Gene Expression Assays and conducted with 40 cycles. Ct (cycle threshold) values were automatically converted to delta Ct on device.

2.7. Statistical analysis

The results of our studies were statistically evaluated in IBM SPSS 20.00 package program. One way ANOVA was used for the significance of the groups, the Tukey test from the multiple comparison test. $p < 0.05$ was considered significant.

3. Results

3.1. Vitality test results

After application of agmatine for 1 h, paclitaxel and cisplatin toxicity were determined and MTT test was applied. According to the results of our study, it was observed that the low dose of agmatine significantly increased the cell viability compared to the control group. It is observed that cell viability decreases significantly in groups with cisplatin toxicity only. In the groups with Cisplatin (C5 and C10) administered 1 h after agmatine; agmatin showed a stronger

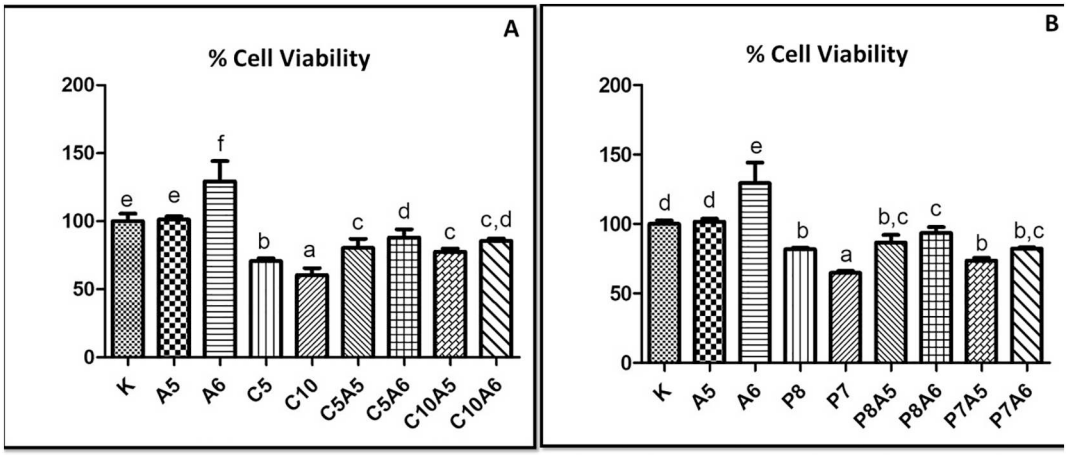

Fig. 1. Cisplatin (A) and paclitaxel (B) 24 h after. Demonstration of the effects of agmatine given 1 h before toxicity on neuronal cell viability by MTT analysis. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. ($p < 0.05$ was considered significant). K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C5: 50 μM Cisplatin, C10: 100 μM Cisplatin, P7: 10^{-7} M Paclitaxel, P8: 10^{-8} M Paclitaxel.

neuroprotective effect at lower dose than the high dose and there was a statistically significant difference between cisplatin groups ($p < 0.05$). (Fig. 1A). In the groups where we create only paclitaxel toxicity, it is observed that cell viability decreases significantly as in the groups of cisplatin. In groups with paclitaxel administered 1 h after agmatine, while low doses of agmatine against neurotoxicity may prevent cell death with either dose, low doses of agmatine have a stronger neuroactive effect in high dose paclitaxel groups ($p < 0.05$) (Fig. 1B).

After 24 h of toxicity with paclitaxel and cisplatin, determined doses of agmatine were administered. MTT test was performed after 1 h. The low dose of agmatine according to Fig. 2A and B again increased cell viability in 1 h compared to the control group. Cisplatin and paclitaxel groups significantly decreased cell viability significantly when compared to the control group but no significant change was observed in cell proliferation after 1 h agmatine administration ($p > 0.05$).

The toxicity of paclitaxel and cisplatin was established and agmatine was applied at the determined doses for 24 h and the MTT test was performed. According to the results of our study, the low dose of agmatine increased cell viability significantly when compared to the control group. It is observed that cell viability decreases significantly in groups with cisplatin toxicity only. After low doses of cisplatin, especially low doses of agmatine increased cell viability significantly ($p < 0.05$). In the toxicity groups created with high cisplatin, both low dose and high dose agmatine increased cell viability significantly ($p < 0.05$) (Fig. It has been observed that cell viability is significantly decreased in only paclitaxel toxicity groups. In paclitaxel-treated groups, agmatine significantly increased cell viability in both doses compared to the high-dose paclitaxel (P7) group. Low dose agmatine administration showed a significant increase in cell viability compared to low-dose paclitaxel (P8) group (Fig. 3B).

3.2. Antioxidant and oxidant results

Neurotoxicity with Paclitaxel; According to the total antioxidant capacity (TAC) and total oxidant capacity (TOC) measurements, in neuron culture medium exposed to agmatine for 1 h and then paclitaxel for 24 h, and there was no significant change in TAC and TOC measurements. However, paclitaxel application increased TOC level and decreased TAC level was significantly improved with low dose agmatine application. However, there was no significant improvement in the

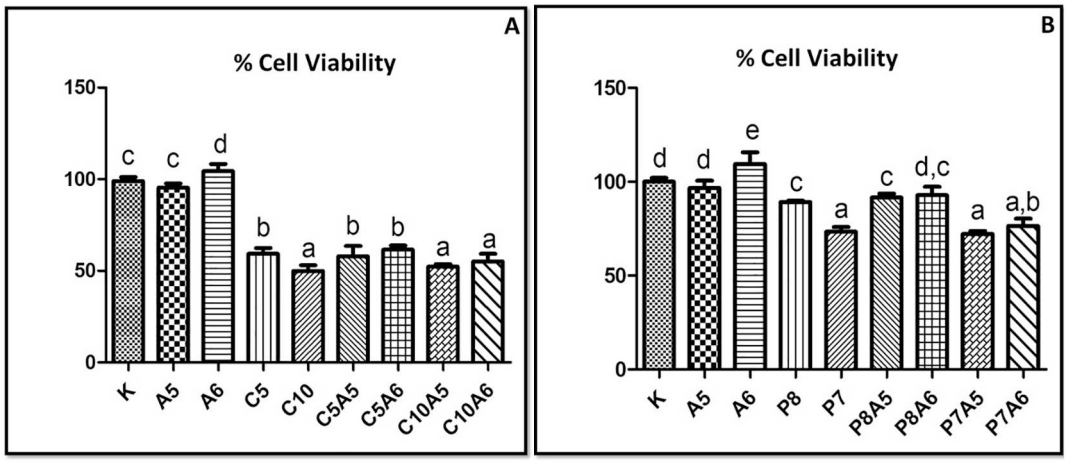

Fig. 2. Cisplatin (A) and paclitaxel (B) 24 h after. Demonstration of the effects of agmatine given 1 h after toxicity on neuronal cell viability by MTT analysis. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. ($p < 0.05$ was considered significant). K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C5: 50 μM Cisplatin, C10: 100 μM Cisplatin, P7: 10^{-7} M Paclitaxel, P8: 10^{-8} M Paclitaxel.

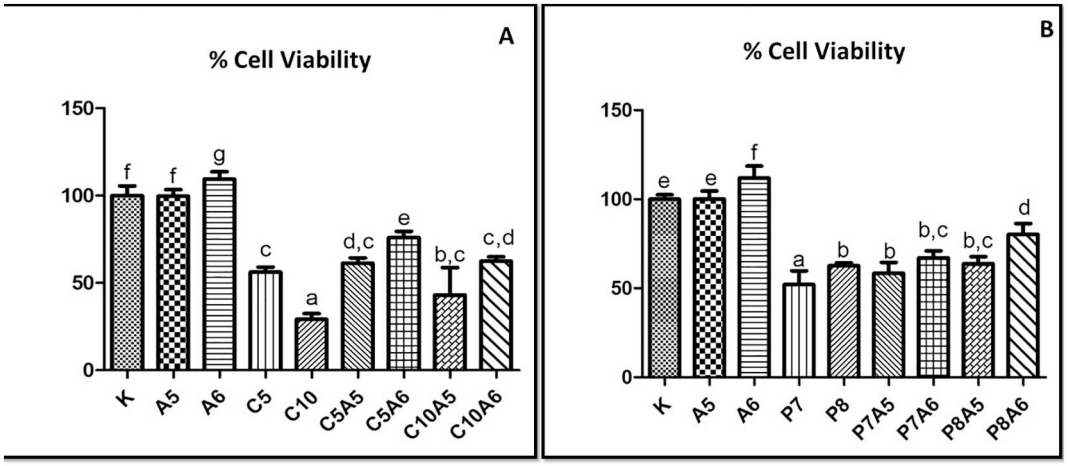

Fig. 3. Cisplatin (A) and paclitaxel (B) 24 h after. Demonstration of the effects of 24 h agmatine exposure after toxicity on neuronal cell viability by MTT analysis. Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. ($p < 0.05$ was considered significant). K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C5: 50 μM Cisplatin, C10: 100 μM Cisplatin, P7: 10^{-7} M Paclitaxel, P8: 10^{-8} M Paclitaxel.

increased TOC level and decreased TAC level in the groups treated with agmatine for 1 h after the toxicity was established. Finally, 24-hour agmatine exposure after toxicity led to significant improvements in both doses with increased TOC levels and decreased TAC levels (Table 1).

Neurotoxicity with Cisplatin; According to the total antioxidant capacity (TAC) and total oxidant capacity (TOC) measurements, in neuron culture medium exposed to agmatine for 1 h and then cisplatin for 24 h, and there was statistically significant decrease in TAC and statistically significant increase in TOC measurements when compared with the control groups. Agmatine application was observed to reverse these changes. The use of agmatine in neuronal Culture Media exposed to 1 h of agmatine after 24 h of cisplatin treatment did not significantly improve the decreased TAC level and increased TOC level due to toxicity. Application of cisplatin in neuronal culture mediums exposed to agmatine for 24 h after treatment with cisplatin for 24 h, decreased TAC levels according to control, while TOC levels increased in cisplatin-treated groups, and the agmatine levels were corrected significantly in favor of these levels (Table 2).

3.3. Inflammation findings

TNF alpha Expression Levels; In our study, cisplatin and paclitaxel groups had significantly increased TNF alpha expression levels compared to the control group. Administration of agmatine 1 h prior brought TNF alpha levels closer to the control group (Fig. 4A). Increased levels of TNF alpha were not affected in the paclitaxel group by agmatine exposure for 1 h after toxicity. However, there was a significant decrease in the levels of TNF alpha in the cisplatin group (Fig. 4B). Decreased levels of TNF alpha were observed with exposure of agmatine 24 h after toxicity (Fig. 4C).

3.4. Apoptosis findings

Caspase 3 Expression Levels; In our study, caspase 3 expression levels increased significantly in cisplatin and paclitaxel groups compared to the control group. It is seen least in the paclitaxel group. The application of agmatinin 1 h ago prior brought the caspase 3 levels closer to the control group again (Fig. 5A). Increased caspase 3 levels were not affected by agmatine exposure 1 h after toxicity (Fig. 5B). A significant decrease in caspase 3 levels was observed with exposure to agmatine 24 h after toxicity (Fig. 5C).

Caspase 9 Expression Levels; In our study, the expression levels of caspase 9 increased in cisplatin and paclitaxel groups compared to the control group. The administration of agmatine 1 h before, brought the caspase 9 levels again back to the control group (Fig. 6A). Exposure of agmatine 1 h after toxicity did not affect increased caspase 3 levels (Fig. 6B). Decreased caspase 9 levels were observed with agmatine exposure 24 h after toxicity (Fig. 6C).

Table 1
The effects of agmatine aganist paclitaxel toxicity on neuronal oxidative system. 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. ($p < 0.05$ was considered significant). TOC: total oxidant capacity, TAC: total antioxidant capacity. K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, P7: 10^{-7} M Paclitaxel, P8: 10^{-8} M Paclitaxel.

Groups	Agmatine exposure for 1 h before toxicity		Agmatine exposure for 1 h after toxicity		24 hours agmatine exposure after toxicity	
	TAC (mmol/L)	TOC (mmol/L)	TAC (mmol/L)	TOC (mmol/L)	TAC (mmol/L)	TOC (mmol/L)
K	2.41 ± 0.62^{d}	2.15 ± 0.45^{a}	2.01 ± 0.32^{b}	2.36 ± 0.51^{a}	2.74 ± 0.51^{c}	2.22 ± 0.24^{a}
a5	2.37 ± 0.38^{d}	2.26 ± 0.56^{a}	2.07 ± 0.35^{b}	2.20 ± 0.44^{a}	2.76 ± 0.44^{c}	2.36 ± 0.35^{a}
a6	2.27 ± 0.51^{d}	2.25 ± 0.31^{a}	2.14 ± 0.29^{c}	2.10 ± 0.38^{a}	2.77 ± 0.51^{c}	2.63 ± 0.37^{a}
p7	1.73 ± 0.44^{a}	4.32 ± 0.37^{d}	1.84 ± 0.27^{a}	3.69 ± 0.48^{b}	1.15 ± 0.48^{a}	4.91 ± 0.50^{d}
p8	1.82 ± 0.48^{a}	4.61 ± 0.54^{d}	1.99 ± 0.19^{b}	3.95 ± 0.62^{b}	1.29 ± 0.62^{a}	4.77 ± 0.45^{d}
P7a5	1.80 ± 0.47^{a}	3.89 ± 0.45^{c}	1.85 ± 0.37^{b}	3.68 ± 0.47^{b}	2.39 ± 0.44^{b}	3.36 ± 0.36^{b}
p7a6	1.98 ± 0.53^{b}	3.52 ± 0.34^{b}	1.94 ± 0.25^{b}	3.63 ± 0.53^{b}	2.31 ± 0.53^{b}	3.08 ± 0.42^{b}
P8a5	2.02 ± 0.39^{c}	3.43 ± 0.23^{b}	1.89 ± 0.29^{b}	3.84 ± 0.39^{c}	2.32 ± 0.38^{b}	3.84 ± 0.35^{c}
P8a6	2.13 ± 0.44^{c}	3,36 ± 0.50^{b}	1.97 ± 0.18^{b}	3.80 ± 0.44^{c}	2.35 ± 0.39^{b}	3.23 ± 0.27^{b}

Table 2
The effects of agmatine aganist cisplatin toxicity on neuronal oxidative system. 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. ($p < 0.05$ was considered significant). TOC: total oxidant capacity, TAC: total antioxidant capacity. K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C5: 50 μM Cisplatin, C10: 100 μM Cisplatin.

Groups	Agmatine exposure for 1 h before toxicity		Agmatine exposure for 1 h after toxicity		24 h agmatine exposure after toxicity	
	TAC (mmol/L)	TOC (mmol/L)	TAC (mmol/L)	TOC (mmol/L)	TAC (mmol/L)	TOC (mmol/L)
K	2.01 ± 0.32^{b}	2.36 ± 0.51^{b}	2.41 ± 0.62^{b}	2.15 ± 0.31^{a}	2.74 ± 0.51^{c}	2.22 ± 0.27^{a}
a5	2.07 ± 0.35^{b}	2.20 ± 0.44^{a}	2.37 ± 0.38^{b}	2.26 ± 0.22^{a}	2.76 ± 0.44^{c}	2.36 ± 0.35^{a}
a6	2.14 ± 0.29^{c}	2.10 ± 0.38^{a}	2.27 ± 0.51^{b}	2.25 ± 0.24^{a}	2.77 ± 0.51^{c}	2.63 ± 0.45^{a}
C5	1.64 ± 0.27^{a}	3.99 ± 0.48^{d}	1.63 ± 0.44^{a}	4.32 ± 0.35 ^{b}	1.02 ± 0.48^{a}	4.91 ± 0.37^{e}
C10	1.69 ± 0.19^{a}	4.05 ± 0.62^{d}	1.72 ± 0.48^{a}	4.61 ± 0.32^{b}	1.09 ± 0.62^{a}	4.77 ± 0.45^{e}
C5a5	2.11 ± 0.37^{c}	2.68 ± 0.47^{c}	1.80 ± 0.47^{a}	4.29 ± 0.45^{b}	2.39 ± 0.44^{b}	3.36 ± 0.36^{c}
C5a6	2.04 ± 0.25^{b}	2.33 ± 0.53^{b}	1.82 ± 0.53^{a}	4.22 ± 0.47^{b}	2.31 ± 0.53^{b}	3.08 ± 0.56^{b}
C10a5	1.99 ± 0.29^{b}	2.44 ± 0.39^{b}	1.92 ± 0.39^{a}	4.43 ± 0.37^{b}	2.32 ± 0.38^{b}	3.84 ± 0.32^{d}
C10a6	2.06 ± 0.18^{b}	2.40 ± 0.44^{b}	1.93 ± 0.44^{a}	4.36 ± 0.32^{b}	2.35 ± 0.39^{b}	3.63 ± 0.35^{d}

4. Discussion

In our study, it was shown that agmatine application before and after toxicity increases the proliferation of neuron cells. It has been observed that agmatine increases cell proliferation, especially at a maximum dose of 10^{-6} M. However, short-term agmatine administration (1 h) after toxicity did not have an effect on proliferation. Biochemical and molecular analyses of oxidant, antioxidant, inflammatory and apoptotic markers have been performed to show which mechanisms of the neurotoxic effect agmatine has shown. The administration of agmatine in uncontrolled and high doses may cause polyamine stress. Polyamine stress has been associated with many mental disorders, in particular suicide [14]. It has now been reported that the use of agmatine as a food supplement by bodybuilders due to its pain relief activities may have some negative effects due to its possible addictive effects [15]. In light of this information, it is observed that the minimum dose and controlled use of agmatine for neuroprotective efficacy are essential.

Paclitaxel, which has become widespread in cancer chemotherapy, can now be used with many chemotherapeutic agents. However, the increased risk of developing drug-related toxicity in cancer patients after treatment has led to an increase in alternative approaches to supporting chemotherapeutic drugs. Although there are many side effects of paclitaxel, neurotoxicity has been shown in studies involving our subject [16]. Paclitaxel, a multi-molecule complex containing a voltage-dependent anion channel, opens mitochondrial permeability transition pore, causing the release of calcium from mitochondria. This calcium-mediated neuronal stimulation is thought to play a role in neurotoxicity. In addition to the structural damage of neurons and changes in DNA levels with recurrent acute excitation attacks, platinum agents are thought to be responsible for neurotoxicity through long-term changes in transmembrane receptors and channels [17].

Cisplatin is a potent antineoplastic agent containing platinum, a heavy metal. This compound selectively and specifically inhibits cell proliferation while also having antibacterial properties [18]. As a result of the stimulation of the expression of the antiapoptotic genes in cancer

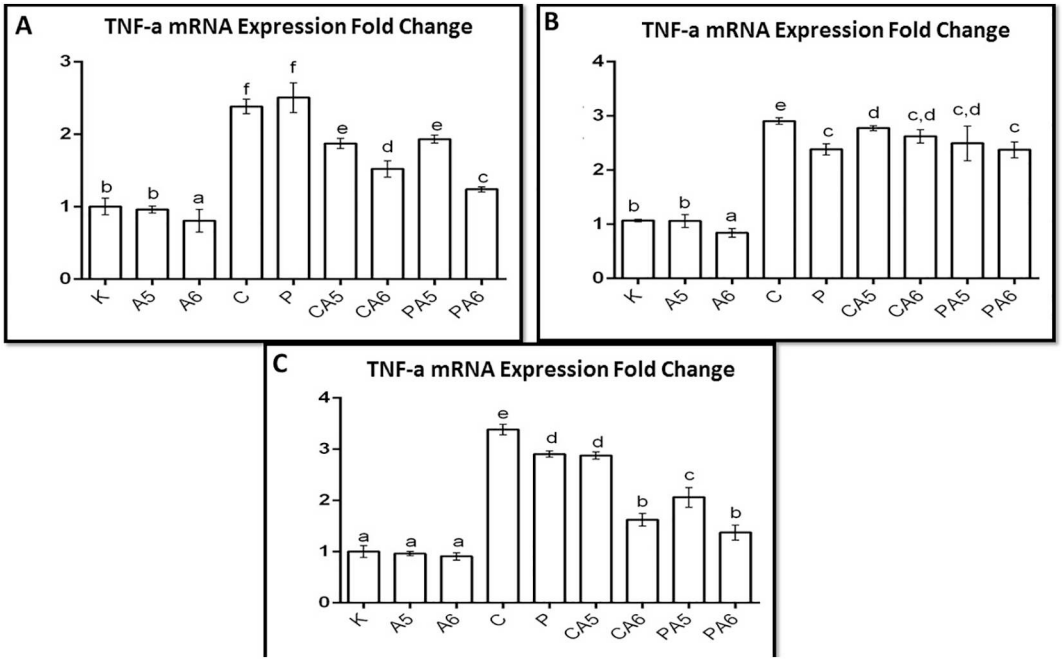

Fig. 4. The effects of agmatine on TNF-a mRNA expression against paclitaxel and cisplatin neurotoxicity. Agmatine exposure for 1 h before toxicity (A), Agmatine exposure for 1 h after toxicity (B) 24 h agmatine exposure after toxicity (C). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. ($p < 0.05$ was considered significant). K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C: Cisplatin, P: Paclitaxel.

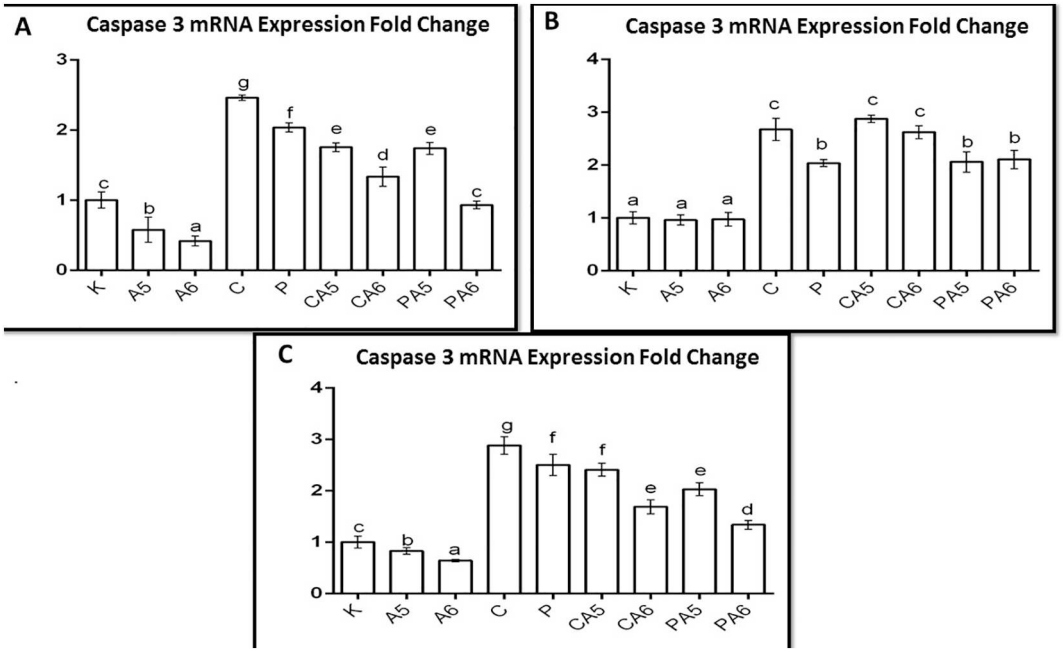

Fig. 5. The effects of agmatine on caspase 3 mRNA expression against paclitaxel and cisplatin neurotoxicity. Agmatine exposure for 1 h before toxicity (A), Agmatine exposure for 1 h after toxicity (B) 24 h agmatine exposure after toxicity (C). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. ($p < 0.05$ was considered significant). K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C: Cisplatin, P: Paclitaxel.

cells, chemoresistance development is shaped with the application of cisplatin [19]. In order to eliminate this resistance, cisplatin should be administered at high doses, but at high doses cisplatin, it causes the most organ/tissue toxicity, such as nephrotoxicity and hepatotoxicity. In lower doses, the effect rate of cisplatin decreases [20]. The most important factor in the toxicity of cisplatin is oxidative stress in mitochondria. As a result, mitochondrial protein-sulfidrylase disappears and mitochondrial membrane potential decreases [21]. Anticancer drugs mainly damage the mitochondria by deterioration of ATPase-dependent Na^+/K^+ pumps and Ca^{+2} homeostasis. The reduction of mitochondrial deterioration or suppression of mitochondrial electron transport chain and ATP synthesis has been shown to alleviate the symptoms of neurotoxicity and demonstrate the important role of mitochondria. The accumulation of dysfunctional mitochondria causes oxidative stress and peripheral nerve damage. In addition, studies have revealed that cisplatin has a toxic effect with a mechanism due to

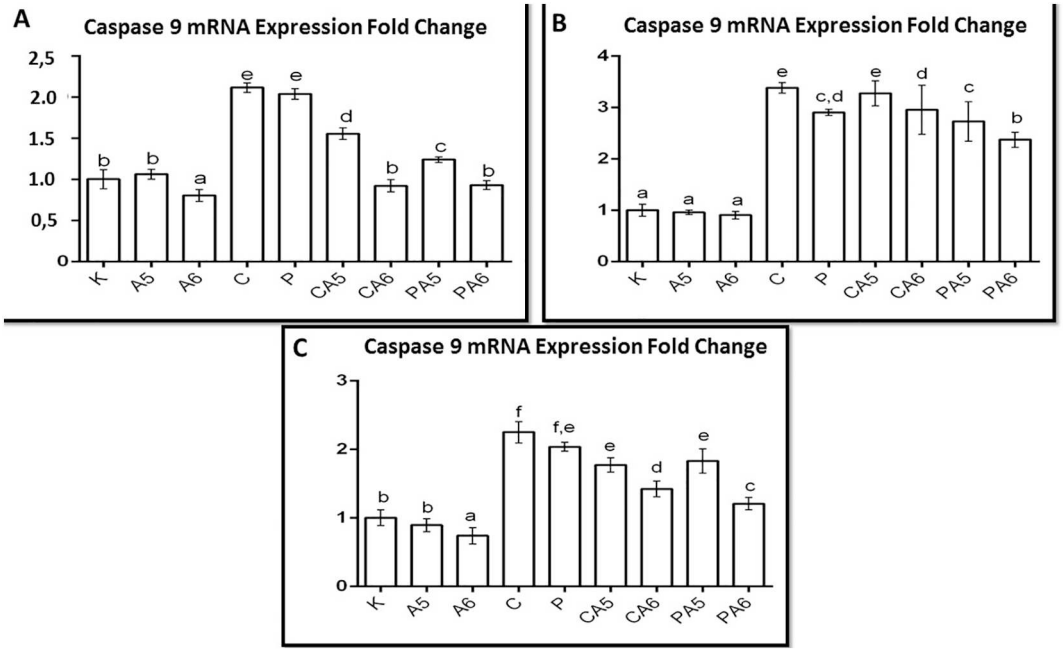

Fig. 6. The effects of agmatine on caspase 9 mRNA expression against paclitaxel and cisplatin neurotoxicity. Agmatine exposure for 1 h before toxicity (A), Agmatine exposure for 1 h after toxicity (B) 24 h agmatine exposure after toxicity (C). Means in the same column with the same letter are not significantly different; means in the same column with different letters indicate significant differences between the groups. ($p < 0.05$ was considered significant). K: Control, A5: 10^{-5} M Agmatine, A6: 10^{-6} M Agmatine, C: Cisplatin, P: Paclitaxel.

oxidative damage. Cisplatin reduces the activity of free radical scavenging enzymes such as SOD, GSH, GSH-Px in tissue and leads to an increase in oxygen radicals, primarily O_2 [22]. Antioxidant compounds have been shown to alleviate this condition. Antioxidants bind to the metabolites of platinum compounds and alkylating agents and protect healthy tissues against cytotoxic effects of chemotherapies [17]. In our study, the agmatine used as a neuroprotectant did not change the antioxidant capacity alone, it significantly improved the toxicity-induced oxidative stress and decreased antioxidant capacity. While no changes in TAC and TOC levels in agmatine administered groups were observed, the study found that it reduced oxidative stress due to anticancer drugs. In our study findings, for neuron culture medium exposed to agmatine for 1 h and then paclitaxel for 24 h, no significant change in TAC and TOC measurements was demonstrated. However, paclitaxel application increased TOC level and decreased TAC level was significantly improved with low dose agmatine application (Table 2). However, there was no significant improvement in the increased TOC level and decreased TAC level in the groups treated with agmatine for 1 h after the toxicity was established with paclitaxel (Table 1). Finally, 24-hour agmatine exposure after paclitaxel toxicity led to significant improvements in both doses with increased TOC levels and decreased TAC levels (Table 1). With cisplatin toxicity, TOC increased and TAC decreased. In neuron culture medium exposed to agmatine for 1 h and then cisplatin for 24 h, there was a statistically significant decrease in TAC and statistically significant increase in TOC measurements when compared with the control groups. Agmatine application was observed to reverse these changes (Table 2). The use of agmatine in neuronal Culture Media exposed to 1 h of agmatine after 24 h of cisplatin treatment did not significantly improve the decreased TAC level and increased TOC level due to toxicity (Table 2). Application of cisplatin in neuronal culture mediums exposed to agmatine for 24 h after treatment with cisplatin for 24 h, decreased TAC levels according to control, while TOC levels increased in cisplatin-treated groups, and the agmatine levels were corrected significantly in favor of these levels (Table 2). In a study conducted with paclitaxel in combination with carboplatin, it was suggested that short-term infusion treatments led to higher rates of neurotoxicity [23]. Another study by Langer et al. shows that short-term treatments are more neurotoxic, as well as that increasing doses of paclitaxel may increase the frequency of neurotoxicity [24]. These findings are supported by our studies. In addition to the absence of the effect of agmatine on TAC and TOC levels in high doses of paclitaxel, the observation of the more toxic effect of higher doses in vitality tests also supports these findings.

When chemotherapy induced damage occurs, a number of inflammatory cells accumulates around the damaged nerves in response to activation of schwann cells and macrophages, and many cytokines and chemokines such as TNF alpha, IL-1β, IL-6, IL are produced. A study by Ledeboer et al. showed an increase in gene expression of proinflammatory cytokine in lumbar dorsal root ganglia treated with paclitaxel. It is known that TNF-α, a cytokine that is implanted in the membrane, has both proinflammatory and extrinsic apoptosis effects in our body [25]. Neurotoxicity studies show that paclitaxel increases the production of TNF-α, a proinflammatory cytokine [26–28]. Agmatin also showed protective activity against glutamate-induced neurotoxicity. Agmatin showed neuroprotective effect on inflammation and apoptotic markers [29]. Our work; administration of agmatine 1 h prior brought TNF alpha levels closer to the control group. Exposure to agmatine 1 h after toxicity did not affect increased TNF alpha levels in the paclitaxel group, but decreased significantly in the cisplatin groups. Decreased levels of TNF alpha were observed with exposure of agmatine 24 h after toxicity (Fig. 4A, B, C). The administration of agmatine 1 h before, brought the caspase 3 levels again back to the level of the control group. Increased caspase 3 levels were not affected by agmatine exposure 1 h after toxicity. A significant decrease in increased caspase 3 levels was observed with exposure to agmatine 24 h after toxicity (Fig. 5A, B, C). The administration of agmatine 1 h before, brought the caspase 9 levels again back to the level of the control group. Increased caspase 3 levels were not affected by agmatine exposure 1 h after toxicity. Decreased caspase 9 levels were observed with agmatine exposure 24 h after toxicity (Fig. 6A, B, C). This shows that paclitaxel can cause inflammation as well as apoptosis of neuronal cells. In our study, caspase 3, 9 and TNF alpha expression levels increased significantly with application of cisplatin and paclitaxel into primary cell culture. The least increase is seen in the paclitaxel group. Increased inflammation and apoptosis due to toxicity were reversed by agmatine administration. These effects of agmatine were not observed in the groups exposed for 1 h after toxicity.

In conclusion, agamatine did not show an acute effect against the toxicity of the disease and did not produce statistical significance in any dose. However, it has been shown to have proliferative efficacy with chronic exposure such as 24 h to toxicity. With toxicity following 1 h of exposure to agmatine (25 h of agmatine in total), proliferative effects have again emerged. In light of these findings, it is clear that agmatine counteracts chronic exposure to neurotoxicity, which is one of the limiting side effects of anticancer drugs. It will be useful to add studies to literature with the hypothesis that agmatine is a proliferative target that can be used in cases resulting in neural death.

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