TÜBİTAK

Turkish Journal of Veterinary and Animal Sciences

http://journals.tubitak.gov.tr/veterinary/

Research Article

Turk J Vet Anim Sci
(2016) 40: 575-582
© TÜBİTAK
doi:10.3906/vet-1510-49

The protective effects of cysteamine, putrescine, and the combination of cysteamine and putrescine on fibrosarcoma induced in mice with 3-methylcholanthrene

Abdullah DOĞAN[1], Pınar AKSU KILIÇLE[2], Dinçer ERDAĞ[3,*], Ali Nazmi Can DOĞAN[4], Kadir ÖZCAN[5], Ertan DOĞAN[6]

[1]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey
[2]Department of Biology, Faculty of Sciences and Arts, Kafkas University, Kars, Turkey
[3]Department of Medical Services and Techniques, Atatürk Vocational School of Health Services, Kafkas University, Kars, Turkey
[4]Department of Internal Medicine, İzmir Bozyaka Research and Training Hospital, Bozyaka, İzmir, Turkey
[5]Department of Pathology, Uşak School of Health, Uşak University, Uşak, Turkey
[6]Göle District Directorate of Food, Agriculture, and Livestock, Göle, Ardahan, Turkey

Received: 15.10.2015 • **Accepted/Published Online:** 27.02.2016 • **Final Version:** 02.11.2016

Abstract: In this study, the protective effects of cysteamine, putrescine, and the combination of cysteamine and putrescine were investigated in mice with fibrosarcoma induced with 3-methylcholanthrene (3-MC). A total of 135 adult male albino mice (*Mus musculus*), 2–3 months old and weighing 20 ± 2.0 g, were used in this study. All groups (each consisting of 15 mice) were fed ad libitum. The experimental groups were Group 1 (drinking water), Group 2 (0.2 mL sesame oil s.c.), Group 3 (1 mg 3-MC/0.2 mL sesame oil), Group 4 (0.1% cysteamine), Group 5 (0.1% putrescine), Group 6 (0.1% cysteamine + putrescine), Group 7 (1 mg 3-MC/0.2 sesame oil + 0.1% cysteamine), Group 8 (1 mg 3-MC/0.2 mL sesame oil + 0.1% putrescine), and Group 9 (1 mg 3-MC/0.2 mL sesame oil + 0.1% cysteamine + 0.1% putrescine). Experimental groups were given cysteamine and/or putrescine mixed with drinking water right after being injected with 3-MC subcutaneously. After 7 months, the mice were euthanized by means of cervical dislocation and were evaluated morphologically and histopathologically. The results showed that the number of tumors of 3-MC-induced fibrosarcoma were reduced from the highest to the lowest by cysteamine, cysteamine + putrescine, and putrescine, respectively.

Key words: Fibrosarcoma, 3-methylcholanthrene, cysteamine, putrescine

1. Introduction

Although many studies have been done on cancer, it is still a disease that is very difficult to treat. Healthy cells as well as cancer cells suffer from the effects of the drugs given in treatment. This increases the toxicity of antitumor drugs (1). In order to achieve effective treatment, it is very important to discover drugs that have a very specific effect.

Chemical substances are one of the major causes of tumors. Substances that enter the body exhibit carcinogenic effects either directly (primary carcinogens) or through their metabolites (secondary carcinogens). Carcinogenic substances show their effect by reacting with DNA. Compounds of methyl or epoxide can bind to DNA and cause cancer. If the tumor cell that develops is not eliminated by the immune system, it grows and turns into cancer (1–3).

Animals like mice, guinea pigs, cats, and dogs are frequently used in cancer research. One of the primary chemical substances used to induce cancer is 3-methylcholanthrene (3-MC), an unsaturated polycyclic aromatic hydrocarbon (PAH). 3-MC is effective at causing DNA methylation. It initiates cancer by causing a breakdown in the DNA molecule. 3-MC administered subcutaneously to mice at a dose of 1 mg has been reported to cause fibrosarcoma at a rate of 70%–80% within 1–2 months (4–7). At a dose of 200 mg/kg, 3-MC causes cancer when administered orally to laboratory animals (4–6). Studies have also determined that 3-MC has genotoxic and immunotoxic effects (8,9).

Epoxide metabolites of 3-MC are highly predisposed to reacting with DNA, resulting in a carcinogenic effect. The metabolites induce oxidation, methylation, or hydroxylation in DNA. These reactions explain the mechanism of 3-MC's carcinogenic effect (1,2,4,5,7). The effect of 3-MC can be observed in monooxygenase, which is an aryl hydrocarbon receptor. In particular, the

* Correspondence: dincererdag@hotmail.com

induction that occurs in monooxygenases that carry flavin causes the speed of the metabolism to increase (10).

3-MC is highly soluble in fat. It spreads well throughout the body and easily passes through natural barriers. It undergoes oxidation and conjugation in the liver and other organs. It is eliminated through bile and enters enterohepatic circulation. These pharmacokinetic characteristics strengthen 3-MC's carcinogenic effect (1,4,11). PAH easily spreads through tissues and passes through the placenta, giving it the potential to cause cancer in the fetus (8).

Biogenic amines like cysteamine and putrescine form through decarboxylation of amino acids in the body. Cysteine, which plays a role in the synthesis of glutathione and polypeptides in the immune system, undergoes decarboxylation in the body and is converted into thioethanolamine, which is also called cysteamine. Since cysteine plays a role in the conjugation of xenobiotics, the balance between cysteine and cysteamine is very important. Cysteamine plays a role in energy production. It enters the structure of coenzyme A (cysteamine + beta-alanine + pantoic acid + adenosine, in turn). It binds to acetic acid, both serving as a buffer and contributing to energy production by transporting it to mitochondria (1,4,5,12,13). Cysteamine is used in the treatment of cystinosis (15 mg/kg) (14,15). Vecsei et al. (13) and Wilmer et al. (15) reported that cysteine reduces the synthesis of somatostatin. This means that cysteamine has the potential for use in growth deficiency. Studies that have been done claim that it is effective against fibrosarcoma (4,5).

Putrescine is a diaminobutane that forms through the decarboxylation of ornithine. It is a foul-smelling product that develops in meat and canned animal food (1,4,5). It is thought that putrescine plays a role in the growth and differentiation of cells. It can be considered to be a storage location for amine in the body (16,17). It has been claimed that it can cause cell growth because it stores amine, but that nitrous oxide derivatives synthesized from amine can cause damage to cells. It has been shown that putrescine is found in plants and that some of its analogues can induce apoptosis. It has been determined that it has an antiproliferative effect because of its role in the oxidative system (18). For this reason, it is thought that putrescine may be effective for the treatment of tumors. Furthermore, it should be noted that ornithine decarboxylase enzyme inhibitors reduce the risk of colon cancer (16).

It can be claimed that, due to the functional groups they carry, cysteamine and putrescine can suppress both energy metabolism and the proliferation rate of cells. Findings obtained in some studies confirm this idea (4,5). The relationships of cysteamine and putrescine to structures like coenzyme A (CoA), flavin adenine dinucleotide (FAD), and nicotinamide adenine dinucleotide (NAD), as well as the relationship of cysteamine and glutathione, lead to the possibility that they could have an effect on proliferation.

The goal of this study was to investigate the effects of cysteamine, putrescine, and the combination of cysteamine and putrescine on fibrosarcoma induced in mice with 3-MC.

2. Materials and methods

This research was conducted after obtaining permission from the local ethics committee for animal experiments at Kafkas University (KAÜ-HADYEK 13.04.2012/04). A total of 135 male albino *Mus musculus* mice aged 2–3 months and weighing 20 ± 2.0 g were used in the research. The mice were separated into 9 groups of 15 animals each. All groups were fed ad libitum with standard diet and water intake. The feed was obtained from the Bayramoğlu feed factory in Erzurum; cysteamine (CAS: 156-57-0) was obtained from Fluka, putrescine (P7505) was obtained from Sigma, and 3-MC (CAS: 56-49-5) was obtained from Supelco. 3-MC was prepared with sesame oil at a dose of 1 mg/0.2 mL, while separate 0.1% solutions of cysteamine and putrescine were prepared with drinking water for a maximum of a 3-day supply. The solutions were given to the mice with drinking water ad libitum. Sesame oil and 3-MC were injected in the dorsal thoracic region of the mice (4,6).

Group 1 was kept as a control group. Group 2 was subcutaneously injected with 0.2 mL of sesame oil, and Group 3 was subcutaneously injected with 0.2 mL of the 3-MC solution. Group 4 was administered 0.1% cysteamine, Group 5 was administered 0.1% putrescine, and Group 6 was administered a mixture of 0.1% cysteamine and 0.1% putrescine for the duration of the experiment. Group 7 was subcutaneously injected with 0.2 mL of the 3-MC solution as well as 0.1% cysteamine via drinking water on the same day for the duration of the experiment. Group 8 was subcutaneously injected with 0.2 mL of the 3-MC solution and was also given 0.1% putrescine via drinking water on the same day for the duration of the experiment. Group 9 was subcutaneously injected with 0.2 mL of the 3-MC solution and was also given 0.1% cysteamine and 0.1% putrescine solutions via drinking water on the same day for the duration of the experiment.

The animals were checked every 12 h and monitored for 7 months. At the end of that period, the animals

were euthanized with cervical dislocation. The dead animals were weighed and their tissue was subjected to morphological and histopathological analyses (19). The sizes and weights of the tumors were measured. Samples from tissue and fibrosarcoma masses were fixed in a formaldehyde-alcohol solution and then embedded in paraffin blocks, from which 6-µm-thick sections were obtained. The sections were stained with hematoxylin and eosin for analysis. Minitab 12.1 was used for statistical evaluation of the tumor data (20).

3. Results

A morphological examination of the mice revealed that no lesions were observed at the injection sites in Groups 1, 2, 4, 5, and 6, while hair loss, dermatitis, and irritation were observed in the location where 3-MC was injected in Groups 3, 7, 8, and 9. Table 1 shows the data for animals that died, survived, and developed tumors during the experiment.

No tumors were observed in the mice in Groups 1, 2, 4, 5, and 6, but 11 tumors developed in Group 3 (73.3%), 7 tumors developed in Group 7 (46.6%), 10 tumors developed in Group 8 (66.6%), and 8 tumors developed in Group 9 (53.3%). Figures 1 and 2 show a mouse in Group 3 that developed a tumor and the dissected fibrosarcoma. The fibrosarcomas were microscopically verified. Figure 3 shows the microscopic findings for a mouse that developed a tumor (Group 3).

Mean weights for the mice were 28.5 g in Group 1, 27.8 g in Group 2, 27.6 g in Group 4, 29.5 g in Group 5, and 28.9 g in Group 6. The weight of the mice and the number, weight, and size of the tumors were measured; these data are presented in Table 2. When the results were statistically evaluated with the chi-square test, no significant difference was found between the groups with regard to the mouse and tumor weights or the number of tumors ($P \leq 0.05$).

4. Discussion

Many studies have been done to develop new drugs that have specificity and can be used in the diagnosis, treatment, and prevention of cancer. The types of animals and the length of the experiment in such studies vary. Mice are generally monitored for their lifetime, while rats and other animals are monitored for 2 years. Sözmen et al. (6) administered 3-MC to cause fibrosarcoma in mice and monitored the animals for 6.5 months. In another study on mice, Doğan et al. (4,5) waited 4–12 months for fibrosarcoma to develop in the mice. In the present study, the animals were monitored for 7 months. 3-MC is widely used to cause cancer for experimental purposes (4–7,9,21). As a PAH, 3-MC causes cancer within 2–4 weeks when administered via oral, intraperitoneal, or subcutaneous routes at doses of 40–200 mg/kg (4–6,21). Tumors have been obtained by subcutaneously administering 3-MC to mice at a dose of 1 mg (4–6).

The effect of 3-MC in the body varies depending on the animal species, individual susceptibility, and the conditions of the experiment. For this reasons, 3-MC causes fibrosarcoma to develop in mice at varying rates (approximately 50%–75%). In a study conducted by Doğan et al. (4), the authors reported that tumors

Table 1. Data for animals in the groups that survived and developed tumors.

Group	N	Total number of animals with tumors	Number of animals with tumors living 7 months later	Number of healthy animals living 7 months later
Group 1 (control)	15	-	-	13
Group 2 (SO)	15	-	-	14
Group 3 (3-MC)	15	11	5*	2*
Group 4 (C)	15	-	-	10
Group 5 (P)	15	-	-	12
Group 6 (C + P)	15	-	-	11
Group 7 (3-MC + C)	15	7	5*	3*
Group 8 (3-MC + P)	15	10	7*	3*
Group 9 (3-MC + C + P)	15	8	5*	3*

SO: Sesame oil, C: cysteamine, P: putrescine. *: The difference between groups was not significant ($P \leq 0.05$).

Figure 1. A mouse with fibrosarcoma belonging to Group 3.

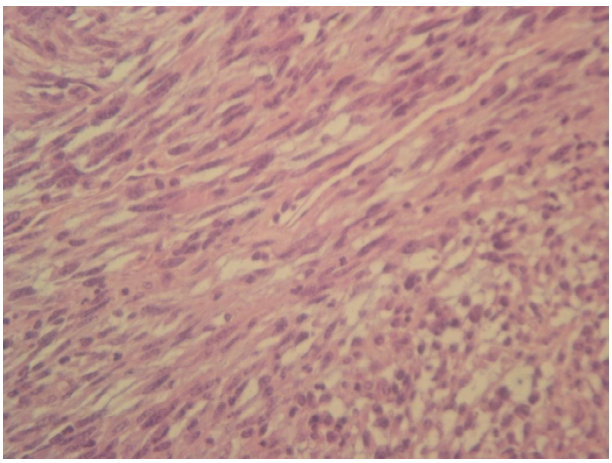

Figure 2. A resected fibrosarcoma belonging to Group 3.

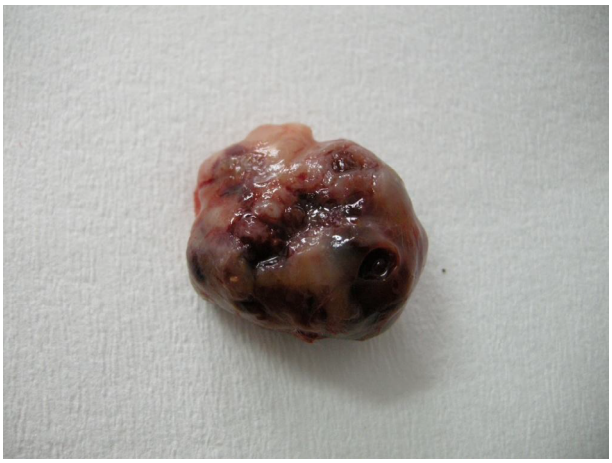

Figure 3. Microscopic view of the fibrosarcoma resected from a mouse belonging to Group 3, 200×.

developed at a rate of 66.6% when 3-MC was administered subcutaneously at a dose of 1 mg. In a different study conducted by Doğan et al. (5), fibrosarcoma was identified at a rate of 73.3% when 3-MC was administered to mice at a dose of 1 mg. In the present study, tumors also developed at a rate of 73.3% using the same method. The tumors were histopathologically verified. The relationship between the dimensions and weight of tumors was determined to be statistically insignificant. No metastatic loci were observed in animals with fibrosarcoma that underwent necropsy. This is not surprising, since it is known that fibrosarcomas do not often cause metastasis. However, it has been reported that after administering 3-MC to mice subcutaneously at a dose of 1 mg, metastasis was observed in the liver, lungs, and kidneys (4,5,21).

Because cysteamine functions in the biosynthesis of CoA, it plays an important role in the tricarboxylic acid cycle (TCA). This enzyme provides a form of cysteine storage for the body. Cysteine that is in storage cannot be used for the synthesis of glutathione. This situation can suppress the detoxification of certain chemical substances, thus increasing their toxicity. When an organism is given cysteamine, the levels of free cysteamine and glutathione increase. Upon administration, cysteamine forms CoA by binding to adenosine compounds such as NAD and FAD, which play a role in energy metabolism. As a result, it can hinder cell proliferation because energy metabolism is negatively affected. For this reason, cysteamine can suppress the effects of carcinogenic agents and can also prevent cells from multiplying. This hypothesis has been supported by many studies. Cysteamine has been found to reduce the incidence of fibrosarcoma induced with 3-MC in mice (4,5). Some studies report that cysteamine has an antimutagenic effect (22). It has also been claimed that it has an antiproliferative effect (23,24). This effect has been demonstrated in erythroblastic leukemia. Its induction of apoptosis has been attributed to the fact that it forms a complex with nitrous oxide (NO) in the cytochrome oxidase enzyme. N-acetylcysteine, a compound of cysteine, inhibits proliferation in cancer caused by 3-MC (25). It has been shown that cysteamine prevents genotoxicity by binding with hydroxylated bases that are carcinogenic (22). Pantetheinase hydrolyzes acetyl-CoA, which stores cysteamine in the body, and releases pantetonic acid and cysteamine. Cysteamine, on the other hand, transforms into cysteine and enters the glutathione structure, thus playing a role in the detoxification of xenobiotics (26). Because cysteamine is part of the structure of CoA, it is a necessary substance for producing energy in the

Table 2. Animal weights and tumor count, weight, and dimensions according to group.

No	G 3			G 7			G 8			G 9		
	AW	TW	TD	AW	TW	TD	AW	TW	TD	AW	TW	TD
1	27.6	6	2 × 2.5 × 2	27.6	-	-	26.8	7.1	3 × 2 × 2	26.2	6.2	3 × 2.5 × 2
2	30.1	5	2 × 2.5 × 2	31.5	-	-	30.4	6	2 × 2.5 × 2	28.2		-
3	33.5	-	-	27.2	8	3 × 3 × 2	25.5	4.4	2 × 2 × 1	26	5.2	2 × 2 × 2
4	27.8	6	3 × 2 × 2	24.8	-	-	30.4	5.1	2.5 × 2. × 2	26.3	-	
5	31.2	-	-	30.2	3.3	2 × 1 × 1	27.2	6	3 × 2 × 2	29.5	6.5	3 × 2 × 2
6	27.7	5.6	2 × 2 × 1.5	27.5	-	-	29	-	-	28.1	-	-
7	26.6	6.7	2 × 2 × 2	30.5	-	-	30.5	6.1	2 × 2.5 × 2	27.1	7	2 × 2.5 × 2
8	25.8	5.8	2 × 2.5 × 1	29.3	7	2 × 2 × 2	33.5	5	2 × 3 × 1	32.4	-	
9	27.6	7.1	3 × 2 × 2	28.7	-	-	28	-	-	36.2	5	2 × 2 × 1.5
10	32.3	-	-	28.2	6	2 × 2 × 2	33.2	-	-	28.5	3.5	2 × 2 × 1
11	32.5	6.5	2 × 2.5 × 2	29,4	7	2 × 3 × 2	33.3	-	-	25.5	-	-
12	20.7	-	-	30.6	5	2 × 3 × 2	29	6.1	2.5 × 2 × 2	30.3	-	-
13	30.6	8.3	2 × 3 × 2.5	26.1	7	3 × 2 × 2	28.5	-	-	29.8	5.8	2 × 2 × 2
14	27.3	7.9	2.5 × 3 × 2	30.4	-	-	28.4	7	3 × 2 × 2	30	7	3 × 2 × 1.5
15	27.1	3.2	1 × 1 × 2	30.4	-	-	30	5.6	2 × 2 × 1.5	30.8	-	-
Mean	28.6	6.2		28.8	6.2		29.6	5.8		29	5.8	

G: Group, AW: animal weight, TW: tumor weight, TD: tumor dimensions. Weight has been given in grams, and dimensions are in cm.

mitochondria. It has been reported that this is why, in cases of cystinosis, energy metabolism breaks down and the ATP level drops (15). In a study conducted by Doğan et al. (4), the authors reported that fibrosarcoma occurred at a rate of 73.3% in the group that was administered 3-MC, but only 53.3% in the group that was given 3-MC immediately followed by cysteamine. Another study done by Doğan et al. (5) reported tumors at a rate of 73.3% in the group that was given 3-MC, 53.3% in the group that was administered cysteamine 1 month after 3-MC, 40% in the group that was administered putrescine 1 month after 3-MC, and 46.6% in the group that was administered cysteamine as well as putrescine 1 month after 3-MC. The present study found that tumors developed at a rate of 46.6% in the group that was given 3-MC and cysteamine at the same time, but 73.3% in the group that was only given 3-MC. The results of this study do not clearly reveal whether cysteamine administered at the same time or cysteamine administered immediately after administration of 3-MC was more effective in protecting against tumors.

It has been claimed that polyamines influence the development and differentiation of cells (16,27). It is known that at low doses, polyamines cause colon cancer to advance (28). However, it has also been claimed that putrescine and its analogues induce apoptosis, thus inhibiting cell multiplication (18,27). It has been determined that high concentrations of putrescine induce apoptosis and prevent proliferation (27). This effect of putrescine is attributed to it increasing NO synthesis, inhibiting redox reactions, or directly binding to carcinogenic agents (18,29). Ornithine conjugates with furan metabolites that have a carcinogenic effect. The fact that ornithine transforms into putrescine

in the body in the presence of ornithine decarboxylase may be a factor in the occurrence of this effect of ornithine (29). These findings strengthen the hypothesis that putrescine may act by binding to carcinogenic agents. Its relationship to coenzyme A and TCA also leads to the conclusion that it has an effect on energy metabolism. It converts alpha-ketoglutaric acid into glutamine via glutamate. It can also be claimed that putrescine binds to acetic acid and blocks energy metabolism, thus leading to an anticarcinogenic effect. This hypothesis is supported by the fact that putrescine is excreted in the urine in the form of products conjugated with acetic acid (5). The study done by Doğan et al. (5) reported tumors at a rate of 73.3% in the group that was given 3-MC, 53.3% in the group that was administered cysteamine 1 month after 3-MC, 40% in the group that was administered putrescine 1 month after 3-MC, and 46.6% in the group that was administered cysteamine as well as putrescine 1 month after administering 3-MC. The study showed that administering putrescine alone provided better protection than administering a combination of cysteamine and putrescine. The present study identified tumors in mice at a rate of 73.3% in the group that was given 3-MC, 46.6% in the group that was given cysteamine, 66.6% in the group that was given putrescine, and 53.3% in the group that was given cysteamine and putrescine together with 3-MC. The study shows that the best protection against fibrosarcoma is provided by cysteamine, followed by the combination of cysteamine and putrescine, and lastly by putrescine alone. The finding that cysteamine and putrescine reduce the incidence of tumors concurs with the other studies, but the percentages of the results were different. It is notable that the differences between the results of the experiments are not statistically significant.

The protective effect of cysteamine may be due to the conjugation it has with other substances as well as the fact that it indirectly plays a role in the formation of chelate and glutathione. Furthermore, biogenic amines convert the alpha-ketoglutaric acid used in TCA into glutamine, potentially disrupting energy metabolism. Administering cysteamine binds adenosine to coenzyme A (cysteamine + beta-alanine + pantoic acid + adenosine). Adenosine structures like NAD and FAD, which play a role in energy metabolism, increase the levels of other free bases used in the synthesis of genetic structures. This may prevent cell proliferation. Putrescine combines with the adenosine base, binding it in the form of CoA (it may bind as putrescine + beta-alanine + pantoic acid + adenosine or putrescine + pantoic acid + adenosine). In the end, it suppresses cell proliferation like cysteamine. CoA binds one acetic acid, while a putrescine compound binds two acetic acids. Aliphatic amines undergo acetylation in the body and are eliminated in the form of acetyl conjugates (1,30). It has been reported that putrescine is eliminated as a conjugate of acetic acid (30). Consequently, putrescine can be expected to provide more protection than cysteamine when taken together with carcinogenic substances. The relationships between biogenic amines and various compounds can be depicted as follows:

Adenosine ⟷ ATP ⟷ NAD ⟷ FAD ⟷ CoA ⟷ genetic bases.

It is apparent that when administration of putrescine is started 1 month after the administration of 3-MC, it affords better protection than putrescine given at the same time as 3-MC. Furthermore, it was found that cysteamine provided better protection in the group in which it was administered immediately after 3-MC, while putrescine provided better protection in the group in which it was started 1 month after 3-MC. This leads us to the conclusion that these results are due to the fact that putrescine prevents energy metabolism (since it conjugates with acetyl). In contrast, it can be claimed that with cysteamine, these effects originate more from the role it plays in the metabolism of xenobiotics (glutathione). A study by Doğan et al. (5) claims that cysteamine and putrescine are comparable when it comes to the formation of CoA. However, the results obtained in the current study do not fully support that hypothesis.

In conclusion, although the results were not statistically significant, it can be said that cysteamine is most effective in reducing the rate of fibrosarcoma induced in mice with 3-MC, followed by the combination of cysteamine and putrescine, and lastly putrescine alone. We are of the opinion that the views proposed in this study are capable of providing a different viewpoint on tumor treatment and the development of new antitumor drugs. In this regard, it is very important that the findings obtained here be verified with more comprehensive studies in order to shed light on those points that are uncertain.

References

1. Doğan A. Toksikoloji Ders Notları. Kars, Turkey: Kafkas Üniversitesi Veteriner Fakültesi, Farmakoloji ve Toksikoloji Anabilim Dalı; 2012 (in Turkish).
2. Forth W, Henschler D, Rummel W, editors. Pharmakologie und Toxikologie. 4. Mannheim, Germany: Völlig Neu Bearbeitete Auflage Bibliographisches Institut; 1983 (in German).
3. Traş B. Genetik toksikoloji. In: Kaya S, Pirinçci İ, Bilgili A, editors. Veteriner Hekimliğinde Toksikoloji. 2nd ed. Ankara, Turkey: Baskı Medisan Yayınları; 2002. pp. 647-670 (in Turkish).
4. Doğan A, Aksu, P, Erdağ D, Bayezıt M, Doğan E, Özcan K. Farelerde 3-metilkolantrenle indüklenen fibrosarkoma üzerine sisteaminin etkileri. Kafkas Üniv Vet Fak Derg 2013; 19: 7-12 (in Turkish).
5. Doğan A, Aksu P, Erdağ D, Özcan K, Doğan E. Farelerde 3-metilkolantrenle indüklenen fibrosarkoma üzerine sisteamin, putresin ve sisteamin-putresin kombinasyonunun etkileri. Kafkas Üniv Vet Fak Derg 2014; 20: 11-19 (in Turkish).
6. Sozmen M, Tunca R, Erginsoy SD. Cyclin A expression is associated with apoptosis and mitosis in murine 3-methylcholanthrene-induced fibrosarcomas. Exper Tox Pathol 2009; 61: 41-49.
7. Sacu D, Bildik A. Deneysel olarak fibrasarkom oluşturulan ratların interlökin 6 (IL-6) ve tümör nekrözis faktör alfa (TNF alfa) düzeylerinin belirlenmesi. Kafkas Üniv Vet Fak Derg 2009; 15: 681-686 (in Turkish).
8. Donovan PJ, Smith GT, Nardone R. The mutagenic effect of 7,12-dimethylbenz[a]anthacene, 3-methylcholanthrene and benzo[a]pyrene to the developing Syrian hamster fetus measured by an in vivo/in vitro mutation assay. Mut Res 2004; 554: 111-117.
9. Lutz CT, Browne G, Petzold CR. Methylcholanthrene causes increased thymocyte apoptosis. Toxicology 1998; 128: 151-167.
10. Celius T, Pansoy A, Mathews J, Okey AB, Henderson MC, Krueger HS, Williams DE. Flavin-containing monooxygenase-3: induction by 3-methylcholanthrene and complex regulation by xenobiotic chemicals in hepatoma cells and mouse liver. Tox Appl Pharm 2010; 247: 60-69.
11. Baijal PK, Fitzpatrick DW, Bird RP. Phenobarbital and 3-methylcholanthrene treatment alters phase I and phase II enzymes and sensitivity of the rat colon to the carcinogenic activity of azoxymethane. Food Chem Toxicol 1997; 35: 789-798.
12. Bentley R. From 'reactive C2 units' to acetyl coenzyme A: a long trail with an acetyl phosphate detour. Trends Biochem Sci 2000; 25: 302-305.
13. Vecsei L, Ekman R, Alling C, Widerlöv E. Influence of cysteamine and cysteine on open-field behaviour, and on brain concentration of catecholamine, somatostatin, neuropeptide Y and corticotropin releasing hormone in the heart. J Neural Transm 1989; 78: 209-220.
14. Basouw M, Levtchenko E. Pharmacokinetics of cysteamine in cystinosis patients treated with hemodialysis. Pediatr Nephrol 2011; 26: 639-640.
15. Wilmer MJ, Kluijtmans LAJ, Van Der Welden TJ, Willems PH, Scheffer PG, Masereeuv M, Monnens LA, Van Den Hauvel LP, Leftchenko EN. Cysteamine restores glutathione redox status in cultured cystinotic proximal tubular epithelial cells. Biochim Biophys Acta 2011; 1812: 643-651.
16. Ruiz-Cano D, Perez-Liamas F, Zamora S. Polyamines, implications for infant health. Arch Argentinos De Pedietria 2012; 110: 244-250 (in Spanish with English abstract).
17. Vargas AJ, Wertheim BC, Gerner EW, Thornson CA, Rock C, Thompson PA. Dietary polyamine intake and risk of colorectal adenomatous polyps. Am J Clin Nut 2012; 96: 133-141.
18. Russo A, Piovano M, Clercuzio M, Lombardo L, Tabasso S, Chamy MC, Vidari G, Cardile V, Vita-Finzi P, Garbarino JA. Putrescin-1,4-dicinnamide from *Pholiota spumosa* (blasidomycetes) inhibits cell growth of human prostate cancer cells. Phytomedicine 2007; 14: 185-191.
19. Luna LG. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd ed. New York: McGraw-Hill; 1968.
20. Minitab. Minitab Release 12.1. State College, PA, USA: Minitab Inc.; 1998.
21. Keshava N. Tumorigenicity of morphologically distinct transformed foci induced by 3-methylcholanthrene in BALB/c 3-T3 cells. Mut Res 2000; 447: 281-286.
22. Grachev SA, Kropachev EV, Litvyakova GI. Synthesis of 5-S-cysteamine-6-hydroxythymine and evidence of its formation in the gamma radiolysis of aqueous solutions of thymine and cysteamine. Izvestiya Akademii Nauk SSSR Seriya Khimicheskaya 1984; 541: 1595-1599 (in Russian with English abstract).
23. Gebhard H. The anticlastogenic effect of various combinations of cysteamine, AET, HCT and amino acids on chromosome damage by trenimon and bleomycin in human lymphocytes in vitro. Hum Genet 1978; 43: 185-203.
24. Sanina NA, Syrtsova LA, Psikha BL, Shkondina NI, Rudneva TV, Kotelnikov AI, Aldoshin SM. Ferrocytochrome c and deoxyhemoglobin in the reaction with the iron cysteamine nitrosyl complex {Fe2[S(CH2)2NH3]2(NO)4}SO4•2.5H2O. Russian Chem B+ 2010; 59: 1994-1998.
25. Juan SH, Lee JL, Ho PY, Lee YH, Lee WS. Antiproliferative and antiangiogenic effects of 3-methylcholanthrene an aryl-hydrocarbon receptor agonist in human umbilical vascular endothelial cells. Europ J Pharm 2006; 530: 1-8.
26. Kaskow BJ, Proffit JM, Blangero J, Moses EK, Abraham LJ. Diverse biological activities of the vascular non-inflammatory molecules: the vanin pantetheinases. Biochem Biopys Res Commun 2012; 417: 653-658.

27. Takao K, Rickhag M, Hegardt C, Oredsson S, Persson L. Induction of apoptotic cell death by putrescine. Inter J Biochem Cell Biol 2006; 38: 621-628.

28. Milovica V, Turchanowa L, Khomutov AL, Khomutow RM, Caspary WF, Stein J. Hydroxylamine-containing inhibitors of polyamine biosynthesis and impairment of colon cancer cell growth. Biochem Pharm 2001; 61: 199-206.

29. Peterson LA, Phillips MB, Lu D, Sullivan MM. Polyamines are traps for reactive intermediates in furan metabolism. Chem Res Toxicol 2011; 24: 1924-1936.

30. Khuhawan MY, Qureshis GA. Polyamines as cancer markers: applicable separation methods. J Chrom B 2001; 764: 385-407.