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RESEARCH ARTICLE

MDA level in colon tissue of the rats with colon cancer model treated with diallyl disulfide

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Dialil disülfit uygulaması yapılan kolon kanser modelli ratların kolon dokusunda MDA seviyesi

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Öz

Amaç: Sunulan çalışma azoksimetan ile kolon kanseri oluşturulan ratlarda sarımsağın biyoaktif bileşiklerinden biri olan dialil disülfid'in, kolon dokusu malondialdehit seviyesi üzerine etkisini araştırmak amacı ile yapıldı.

Gereç ve Yöntem: Çalışmada 58 adet erkek Wistar Albino rat kullanıldı. Hayvanlar 5 gruba ayrıldı. 1. grup kontrol grubu (n=12), 2. grup azoksimetan (15mg/kg vücut ağırlığı) uygulanan kanser grubu (n=12), 3. grup sadece dialil disülfit (DADS, 50 mg/kg vücut ağırlığı) uygulanan (n=11), 4. grup azoksimetan ve dialil disülfit uygulanan (n=11) ve 5. grup ise taşıt madde olan mısır yağının (1ml/kg vücut ağırlığı) verildiği gruptur (n=12). Deneme süresi olan 18 haftanın sonunda servikal dislokasyon ile hayvanlar sakrifiye edildi ve kolon dokuları çıkarıldı. Alınan dokuların homojenizasyonu sağlandı ve ELISA cihazı ile kite uygun prosedür kullanılarak MDA seviyeleri ölçüldü.

Bulgular: Dokularda tümör oluşumu makroskobik olarak tespit edildi. Yapılan analiz sonucu gruplar arasında MDA seviyesi yönünden herhangi bir fark bulunmadığı belirlendi (P>0.05).

Öneri: Sonuç olarak çalışma koşullarında rat kolon dokusunda DADS veya azoksimetan uygulanasının lipid peroksidasyonuna neden olmadığı ifade edilebilir. DADS veya AOM'nin kolon dokusundaki etkinliği, hayvanlara uygulanma şekli ve miktarı göz önüne alınarak ileride yapılacak olan çalışmalara ihtiyaç vardır.

Anahtar kelimeler: AOM, dialil disülfid, kolon, MDA, rat

Abstract

Aim: This study was conducted to investigate the effect of diallyl disulfide, one of the bioactive compounds of garlic, on the malondialdehyde level of colon tissue in rats with azoxymethane-induced colon cancer.

Materials and Methods: A total of 58 male Wistar Albino rats were used in the study. The animals were divided into 5 groups. The first group was the control group (n=12), the second group was the cancer group (n=12) treated with azoxymethane (15 mg/kg body weight), the third group was the group to whom only diallyl disulfide (DADS, 50 mg/kg body weight) was administered (n=11), the fourth group was the group to whom azoxymethane and diallyl disulfide were administered (n=11) and the fifth group was the group to whom corn oil (1 ml/kg body weight) was administered as carrier substance (n=12). The experimental the one which period at the end of 18 weeks, the animals were sacrificed by cervical dislocation and their colon tissues were excised. The tissues were homogenized and MDA levels were measured with the ELISA device using the procedure, suitable for the kit.

Results: Tumor formation in the tissues was macroscopically identified. The analysis results indicated no difference between the groups with respect to their MDA levels (P>0.05).

Conclusion: In conclusion, it can be asserted that the administration of DADS or azoxymethane did not cause lipid peroxidation in the rat colon tissue under the experimental conditions. There is a need for further studies investigating the effectiveness of DADS or AOM in colon tissue by taking the method and the dose of treatment to the animals into consideration.

Keywords: AOM, colon, diallyl disulfide, MDA, rat

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Introduction

Garlic contains sulfur compounds, enzymes, minerals, vitamin (A, B, and C), fiber, water, and 17 amino acids (Omar and Al-Wabel 2010, Tsai et al 2012). Allicin is one of the most active (biologically) compounds in garlic (Gebreyohannes and Gebreyohannes 2013). Garlic contains a rich source of organosulfur compounds (Tsai et al 2012). Organosulfur compounds contain sulfur, an organic compound with beneficial anti-inflammatory, antioxidant and anti-cancerous effects (Al-Ishaq et al 2020). For example, sulfur compounds in garlic are one of the ways to prevent malignant transformation after the administration of chemical carcinogens (Lamm and Riggs 2018). It is also reported that some of the organosulfur compounds in garlic inhibit carcinogen activation, accelerate phase II detoxification processes, induce apoptosis, enhance histone acetylation, and modulate cellular redox status (Yi and Su 2013).
DADS is an organosulfur compound found in garlic. It protects against oxidative stress and displays anti-cancer properties against some molecules such as nitrosamines (Singh 1996, Tapiero et al 2004, Zhou and Mirvish 2005), and is one of the main components of garlic, which is reported to exert significant anticancer effects in many types of cancer (Yin et al 2017, Xie et al 2018). It is also stated that DADS is that might be a potential anti-cancer drug (Yi and Su 2013, Kim et al 2014).

Lipids are the most sensitive biological structures against the toxic effects of reactive oxygen species (ROS). Polyunsaturated fatty acids in the cell membrane react with ROS, and peroxidation occurs (De Zwart et al 1999, Valko et al 2006). Lipid peroxidation is an oxidative process induced by free radicals derived from oxygen in all cells and tissues. This is a complex event defined as the reaction of polyunsaturated fatty acids in cell membrane phospholipids with oxygen radicals to form lipid hydroperoxides. Peroxidation results in disruption of the membrane structure, impairment of the ion gradient of the cell, and increase in permeability and tissue damage. In case of damage of unsaturated fatty acids that maintain membrane fluidity, fluidity is also impaired (Gutteridge 1995, De Zwart et al 1999, Juan et al 2021). Moreover, when membrane damage occurs, this would be irreversible (Juan et al 2021). The most abundant product of lipid peroxidation are 4-hydroxy-2-nonenal (HNE) and malondialdehyde (MDA) (Pizzimenti et al 2013). Lipid peroxidation level varies by tissues. It is reported to appear primarily in liver and in many other tissues such as heart, brain, and kidney (Rikans and Hornbrook 1997).
Malondialdehyde (MDA) is one of the indicators of lipid peroxidation. It leads to negative consequences such as disruption in membrane structure, change in enzyme activity, and impairment of ion exchange through cell membranes. They can also affect protein synthesis and enzymatic events in the cell and cause DNA damage as they can easily pass through the cell membranes. Therefore, MDA is reported to be a mutagenic, genotoxic and carcinogenic compound (Botsoglou et al 1994, De Zwart et al 1999, Niedernhofer et al 2003, Ayala et al 2014, Ito et al 2019). Monitoring of MDA levels, easily identifiable in biological structures and used to assess oxidant damage, can be utilized to monitor lipid peroxidation in various health disorders (Van Bebber et al 1989, De Zwart et al 1999, Hatipoglu and Keskin 2022). Malondialdehyde analysis in tissue and plasma samples is considered beneficial to determine the level of oxidative stress (Van Bebber et al 1989, Mohideen et al 2021). Nevertheless, since samples contain low levels of MDA, it is important that the methods used for its detection is precise (Botsoglou et al 1994, Ito et al 2019, Mohideen et al 2021).
This study aimed to investigate the effect of AOM and DADS on the MDA level of colon tissue.

Material and Methods

Material

This study was derived from a part of the first author's PhD thesis. The study was conducted at the Experimental Medicine Research and Application Centre (SUDAM) of Selcuk University. The ethical approval (2020/51) was obtained from SUDAM. The Scientific Research Projects (SUBAP) unit of Selcuk University funded the study (project number 20112006).

In this study, a total of 58 male Wistar Albino rats (12 weeks old, weighing 240-260 g) were used as animal material. The rats were kept in rooms with adjusted temperature and light. They were fed with feed and water ad libitum during the experimental period (18 weeks). The rats were divided into following 5 groups. Group 1 (Control Group, n=12): They were fed a standard rat diet. Group 2 (Azoxymethane Group, n=12): Standard rat diet + 15mg/kg body weight AOM was injected s.c. for 2 weeks (once a week) (Er et al 2019). Group 3 (Diallyl Disulfide Group, n = 11): Standard rat diet + 50 mg/kg body weight DADS was administered via gavage for the last 3 weeks (5 days per week) (Somade et al 2019). Group 4 (Azoxymethane+ Diallyl Disulfide Group, n = 11): Standard rat diet + 15mg/kg body weight AOM was injected s.c. for 2 weeks (once a week), and 50 mg/kg body weight DADS was administered via gavage for the last 3 weeks (5 days per week). Group 5 (Corn Oil Group, n = 12): Standard rat diet + corn oil was administered via gavage for the last 3 weeks (5 days per week).

Method

DADS and AOM were purchased from Sigma Aldrich. DADS was dissolved in corn oil and AOM was diluted in physiological saline solution. All chemicals were prepared just before the procedure. AOM;15 mg/kg body weight and DADS; 50 mg/

kg body weight were administered. The latest body weight of each animal was measured and administrations were made accordingly. Azoxymethane was subcutaneously injected at a dose of 15mg/kg body weight (once a week for two weeks) in order to generate a colon cancer model. DADS (50 mg/kg body weight) was administered to the animals via gavage for the last three weeks (5 days a week) to assess its effectiveness. The experiment lasted for a total of 18 weeks. The rats in all the groups were fed with feed and water ad libitum during the experimental period (18 weeks). All the animals were euthanized 24 hours after the last dose of gavage.

Colon tissues were excised from the animals that underwent cervical dislocation, the colon tissues were weighed on ice and homogenized in 50 mM phosphate buffer saline solution (pH:7.4) at a ratio of 1:9 by providing appropriate storage conditions. The homogenized samples were centrifuged at +4°C 5000xg for 5 min in accordance with the procedure of the relevant ELISA kits. The supernatants were collected and measured using 96-well ready-made enzyme assay plates with a range of 0.05-10 nmol/ml and precise to 0.01 nmol/ml with catalogue number E0156Ra of Bioassay Technology Laboratory. Values were measured with Biotek brand ELISA (ELx800) device.

Statistical analysis

All statistical analyses were performed using with SPSS 22 software. Data were analyzed by one-way ANOVA. Tukey's test was applied to determine differences between the study groups. Arithmetic means and standard deviations (mean±SD) were calculated.

Results

After the excision of the colon tissue, the polyps were identified in the AOM (Figure 1) and AOM+DADS (Figure 2) groups.

Figure 3 shows the MDA levels of colon tissue in the control, cancer group treated with AOM, and the groups administered with DADS, AOM+DADS and corn oil used as solvent. When compared with the control group, it was observed that AOM and DADS treatments had no statistically significant effect on the MDA levels of colon tissue in rats for 18 weeks ($p>0.05$, Figure 3).

Discussion

Studies have shown that MDA, one of the products of lipid peroxidation, is found in rat colon tissue and has biological properties that may be related to carcinogenesis. Lipid peroxides and their products are involved in various disease processes, including cancer. Many studies have

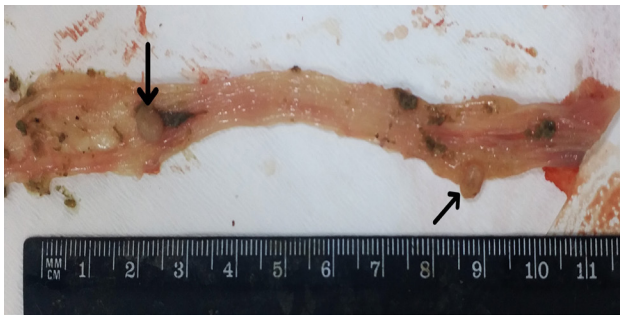

Figure 1. Polyp formation in the colon (arrows), AOM group.

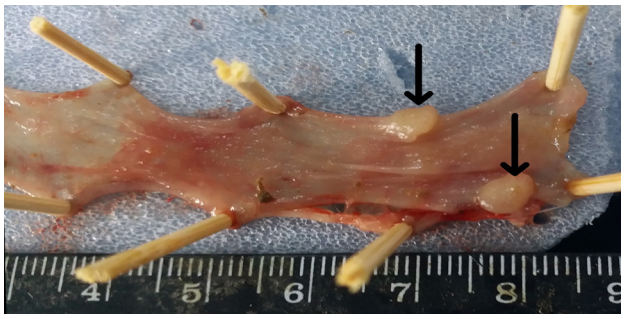

Figure 2. Polyps in the colon (arrows), AOM+ DADS group.

also investigated the possibility of a link between lipid peroxidation and cancer. Regardless of a single cellular or physiological phenomenon, the associated mechanisms should be controlled in order to study the occurrence and development of cancer, and the use of substances with such mechanisms of action is extremely important. Therefore, in this study, DADS, benefits of which have been proven through researches, was administered to male rats with colon cancer models via gavage and it was aimed to investigate its effect.

Chang et al (2008) reported that the serum MDA level was lower in patients with colon cancer compared to the control group. Waly et al (2014) created a colon cancer model by administering an intraperitoneal injection of AOM to 4-week-old male Sprague-Dawley rats and reported that MDA level group statistically significantly elevated in the cancer group compared to the control in the measurements of colon tissue homogenates. When the MDA level in colon tissue was analyzed, a significant elevation was reported in the azoxymethane group compared to the control group (Almagrami et al 2014). In their study, Uyar et al (2021) reported a statistically significant elevation in MDA level in colon tissue samples of the cancer group treated with azoxymethane compared to the animals in the control group. In the present study, no statistically significant difference ($p>0.05$) was observed in the MDA level in the cancer group compared to the control group. Consequently, the lack of MDA change in rats suggested that DADS or AOM treatment did not induce lipid peroxidation under those conditions or the lack of MDA change may be caused by the sufficient antioxidant capacity of the rats. Moreover, the

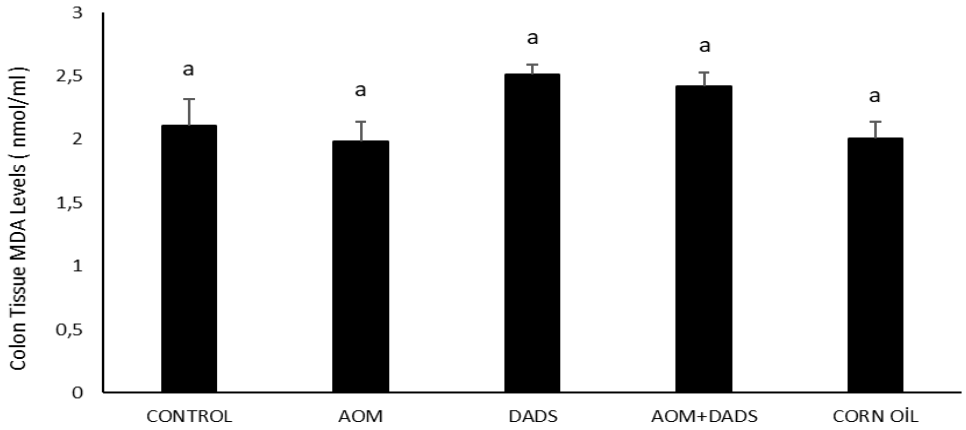

Figure 3. The MDA levels of colon tissue (mean±SE). CONTROL: Healthy control, AOM: Cancer control, DADS: Healthy DADS, AOM + DADS: Cancer + DADS, Corn oil: Solvent. There was no statistical difference between the groups in terms of MDA level (P>0.05).

lack of MDA change in rats may also associated with with the administration frequency of AOM; for example, in their study, Uyar et al (2021) administered AOM of 10 doses for 10 weeks and reported that a change was observed in MDA; on the other hand, in the present study, this was thought to be caused by administration of AOM in 2 doses for 2 weeks.

Although Al-attar et al (2016) reported in their study that blood MDA levels did not differ in the control and corn oil groups, another study (Yaqoob et al 2022) reported a statistically significant ($p<0.05$) elevation in the MDA level of liver tissue in the corn oil group. They stated that this confirmed the effect of corn oil on lipid peroxidation. Haggag et al (2014) reported that feeding with corn oil caused significant elevations in liver and blood MDA levels, significantly increased lipid peroxidation, challenged the antioxidant defense system and may increase the sensitivity of tissues to the breakdown products of lipid peroxides. When compared to the existing studies, there was no statistical difference ($p>0.05$) in the MDA levels of the colon tissue of the corn and control groups in the present study. It was found that the corn oil used did not affect the MDA level in the colon tissue ($p>0.05$). It should be taken into consideration that the differences found between the studies may be due to the administration method and the tissues utilized.

Somade et al (2019) analyzed the MDA levels in kidney tissue of the control, corn oil, and DADS groups in their study and reported no statistical difference between the groups. The study by Kim et al (2014) reported no difference between the control and diallyl disulfide groups in terms of the MDA level in the urinary bladder. Hasan et al (2020) reported no difference in the MDA level between the liver tissues of the control and diallyl disulfide groups. Hassanein et al (2021) reported no difference in the MDA level of kidney tissue between control and DADS (50mg/kg dose for 10 days by gavage) groups in their study involving Wistar albino rats.

Similar to the existing studies, this study revealed that there was no statistical difference ($p>0.05$) in the MDA level of colon tissue of the control, DADS, and corn groups. It was found that the administration of DADS did not change the MDA level of colon tissue for better or worse.

Conclusion

Each study shows that the results may vary depending on the differences in the dose of DADS used, the administration method, the disease studied, and the tissue used. Also, it is necessary to take into consideration that DADS is rapidly metabolized after being absorbed into the body. There is a limited number of studies investigating the effectiveness in colon tissue by creating a colon cancer model with AOM or as a result of DADS application. Therefore, we believe that this study would contribute to similar studies concerning both the model and the results.

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Conflict of Interest

The authors declare no conflict of interest.

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Author Contributions

Motivation/Concept:, ZB; Design: ZB, BSA; Control/ Supervision: ZB; Data Collection and/or Processing: ZB, BSA; Analysis and/or Interpretation: ZB, BSA; Literature Review: BSA; Writing the Article: ZB, BSA; Critical Review: ZB

Ethical Approval

The study was conducted at the Experimental Medicine Research and Application Center (SUDAM) of Selçuk University and the ethical approval (2021/51) was obtained from SUDAM.