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Evaluation of Genotoxic and Cytotoxic Activity of Ganoderma Lucidum In Rat Bone Marrow Induced By Lipopolysaccharide

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Abstract

Objective: It was aimed to investigate the antigenotoxic effect of Ganoderma lucidum against the damage caused by lipopolysaccharide (LPS) in rat bone marrow cells by micronucleus test and comet analysis.

Method: In the experiment, 28 Wistar albino female rats were used and 4 groups were formed. Only physiological saline was applied to the control group. A single dose of 7.5 mg/kg LPS was administered intraperitoneally to the LPS group. LPS+ Ganoderma lucidum (GL): Ganoderma lucidum was given by gavage at a dose of 50 mg/kg per day for one week. Then, on the 8th day, LPS was applied intraperitoneally. Ganoderma lucidum group: GL was given by gavage at 50 mg/kg per day for 1 week. After 24 hours, the rats were euthanized by cervical dislocation under anesthesia. Analyzes were performed for percentage of micronucleated polychromatic erythrocytes (MNPCE) and Polychromatic erythrocytes/Normochromatic erythrocytes (PCE/NCE) ratio and DNA damage in rat femur bone marrow cells.

Results: When the percentage of micronucleus and PCE/NCE ratio were examined, no statistical significance was observed when the groups were compared both with the control and among themselves. Compared to the control group, it was observed that the comet parameters of the experimental groups (excluding the head DNA) increased significantly. Length tail (L tail), tail DNA, olive tail moment (OTM) values were significantly different both compared to the control and among themselves. While there was a significant difference between LPS+GL group and LPS and GL groups in Tail Moment (TM) value and Length comet (L comet) value, no difference was observed between LPS and GL groups.

Conclusion: When all of the findings is considered, Ganoderma lucidum cannot be classified as a genotoxic agent. We can say, however, that it reverses the genotoxic damage in issue.

Keywords: LPS, Ganoderma lucidum, Micronucleus, Comet assay

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Lipopolisakkarit ile İndüklenen Sıçan Kemik İliğinde Ganoderma Lucidum'un Genotoksik ve Sitotoksik Aktivitesinin Değerlendirilmesi

Öz

Amaç: Sıçan kemik iliği hücrelerinde lipopolisakkaritin (LPS) oluşturduğu hasara karşı Ganoderma lucidum'un antigenotoksik etkisi mikronükleus testi ve komet analizi ile araştırılması amaçlanmıştır.

Yöntemler: Deneyde 28 adet Wistar albino dişi sıçanlar kullanılarak ve 4 grup oluşturuldu. Kontrol grubuna sadece serum fizyolojik uygulandı. LPS grubuna, intraperitoneal olarak tek doz 7.5 mg/kg LPS uygulandı. LPS+ Ganoderma lucidum (GL): Ganoderma lucidum bir hafta süreyle günde 50 mg/kg dozda gavaj yoluyla verildi. Daha sonra 8. gün intraperitoneal olarak LPS uygulandı. Ganoderma lucidum grubu: GL 1 hafta boyunca günde 50 mg/kg olacak şeklide gavaj ile verildi. 24 saat sonra sıçanlar anestezi eşliğinde servikal dislokasyonla ötanazi edildi. Sıçan femur kemik iliği hücrelerinde, mikronükleuslu polikromatik eritrositlerin (MNPCE) yüzdesi, Polikromatik eritrositlerin/Normokromatik eritrositlere (PCE/NCE) oranı ve DNA hasarı için analizler yapıldı.

Bulgular: Mikronükleus yüzdesi ve PCE/NCE oranı incelendiğinde gruplar hem kontrol ile hem de kendi aralarında kıyaslandığında isatatistiksel olarak anlamlılık gözlenmedi. Kontrol grubuna göre deney gruplarının kuyruklu yıldız parametreleri (baş DNA hariç) önemli derecede arttığı görüldü. Kuyruk uzunluğu (L tail), kuyruk DNA, olive tail moment (OTM) değerleri hem kontrole göre hem de kendi aralarında anlamlı derecede farklıydı. Kuyruk momenti (TM) değeri ve comet uzunluğu (L comet) değerinde LPS+GL grubu ile LPS ve GL grupları arasında anlamlı fark bulunurken, LPS ve GL grupları arasında fark gözlenmedi.

Sonuç: Tüm bulgular değerlendirildiğinde Ganoderma lucidum genotoksik ajan olarak sınıflandırılamaz. Ancak söz konusu genotoksik hasarı tersine çevirdiğini söyleyebiliriz.

Anahtar kelimeler: LPS, Ganoderma lucidum, Mikronükleus, Comet analizi.

INTRODUCTION

The most powerful microbial mediator linked to the pathophysiology of septic shock and sepsis is lipopolysaccharide (LPS)[1]. It is used in experimental studies in a range ranging from modeling local inflammation to septic shock modeling, depending on the dose and application method[2]. LPS induces septic shock in large doses, but can also cause a well-defined inflammatory state at low concentrations[3].

In order to increase the success of standard treatments and to eliminate undesirable results, some drugs are developed or herbal agents are widely used to support treatment. Ganoderma lucidum, also known as lingzhi in China, yeongji in Korea, and reishi in Japan, is a medicinal mushroom. For thousands of years, these fungi have been utilized to cure and prevent a variety of ailments[4]. Studies have shown that the compounds it contains have very serious medical effects[5]. The major pharmacologically active chemical components of Ganoderma lucidum are polysaccharides and triterpenes, which have garnered a lot of attention for their anti-tumor, anti-angiogenic, anti-hypertensive, anti-complement, and anti-HIV pharmacological activities[6].

Recently, antigenotoxicity and cytotoxicity studies using different materials have become very popular. It is noteworthy that many different plant extracts are used for this purpose in the literature. Examples of commonly used methods for detecting genotoxicity are the Ames test, tests that measure DNA strand breaks in cells (comet test) and cytogenetic tests (chromosomal abnormality and micronucleus test)[7].

Micronuclei (MNs) are defined as formations that arise during mitosis of the cell, are not included in the main nucleus, and originate from whole chromosomes or acentric chromosome fragments[8]. This technique, which is widely used, can be applied both in vivo and in vitro, thanks to its advantages such as easy application, counting more cells and obtaining more statistically significant results in the detection of cytogenetic damage[9]. The MN method is also used as a marker to identify genetic damage caused by toxic agents or to determine the biocompatibility of new products

such as nanoparticles and drugs[10]. The application of various chemicals (strong mutagens and carcinogens) causes chromosome damage or inhibition of mitosis during the division of hematopoietic cells[11].

The International Conference on Harmonization (ICH) guidelines on genotoxicity testing recommended combining the in vivo comet test with the in vivo MN test, as the practice of genotoxicity testing so far has not been able to fully detect all genotoxic aspects of a single test[12]. The comet assay, or single-cell gel electrophoresis, is a simple and sensitive method for measuring DNA damage at the single-cell level. The comet assay can detect DNA double-strand breaks, single-strand breaks, alkali-labile regions, DNA-DNA/DNA-protein cross-links, and incomplete excision repair sites under neutral or alkaline conditions[13].

It is very important to identify substances that will reduce or even minimize the negative effects of LPS, which causes acute inflammation. In this study, it was aimed to determine whether Ganoderma lucidium has antigenotoxic effects on the genotoxicity of lipopolysaccharides in rat bone marrow cells.

METHODS

The rats used in the study were raised in Erciyes University Hakan Çetinsaya Experimental and Clinical Research Center (DEKAM). Ethics committee approval was obtained and 28 Wistar albino female rats, 8 weeks old, weighing between 200-250 g, were used in the study. They were kept in rooms specially prepared for rats, automatically acclimatized in 12 hours of light/dark periods and at a constant temperature of 19-21ºC. Rats were free-fed with normal pellet chow and water. The dose of the substances to be administered was determined according to the weight of the rats. In the study, Lipopolysaccharide (Escherichia coli -serotype O55:B5; Sigma-AldrichCo., St. Louis, MO, USA) was given to the group in which endotoxemia was to be created. The study groups designed for the experiment were formed as follows.

1. Control: 1cc of physiological saline was applied once a day during the experiment.

2. Lipopolysaccharide (LPS): 7.5 mg/kg Lipopolysaccharide (LPS) was dissolved in physiological saline and administered a single dose intraperitoneally (i.p).

3. LPS + Ganoderma lucidum (GL): Ganoderma lucidum was given by gavage at a daily dose of 50 mg/kg for one week. Then, on the 8th day, a single dose of LPS was application intraperitoneally.

4. Ganoderma lucidum (GL): Ganoderma lucidum was given by gavage at 50 mg/kg daily for 1 week.

All applications were done at the same time of day. Rats were sacrificed 24 hours after the last administration[14].

After anesthesia with ketamine hydrochloride (50 mg/kg i.p.) and 2% xylazine hydrochloride (10 mg/kg i.p.) at the end of the study, the animals were sacrificed. The femoral bone of each rat was removed and the bone marrow was collected into an eppendorf tube.

Micronucleus Test

The femurs of the rats were removed by cervical dislocation method and transferred to the centrifuge tube with an injector containing 2 ml of fetal calf serum in the bone marrow of the femur. The bone marrow sample was centrifuged at 2000 rpm for 7 minutes and the supernatant was discarded. The remaining part was suspended with 0.5 ml fetal calf serum and spread on clean slides with the help of a second slide. After drying, it was fixed with methanol for 10 minutes and dried thoroughly. Finally, it was stained with 20% Giemsa for 30 minutes, washed with distilled water and left to dry in the open air. One day after staining, the

preparations were coded by sealing with entellan. 4 preparations were prepared from each rat bone marrow[8].

Genotoxic and antigenotoxic activity, under the microscope (Nikon microscope), 1000 PCE were counted randomly in smears prepared from each rat in all groups at X100 magnification, the MNPCE numbers in them were determined and their percentages were calculated. In addition, to confirm the presence of cytotoxicity, the PCE/NCE ratio was determined by counting a total of 2000 erythrocytes (PCE and NCE). After staining, PCEs were seen as blue and NCEs as orange/pink.

Assessment of DNA Damage with Comet Assay

The comet assay was used to assess bone marrow DNA damage. The comet assay was performed in a neutral environment. Images of 50 nuclei selected at 200x magnification were recorded in a fluorescent microscope (Olympus, BX51, Tokyo, Japan) and then analyzed using the Comet Assay Software Project (CASP-1.2.2, Windows 2010). Seven parameters (head length, tail length, comet length, head DNA, tail DNA, tail moment, and olive tail moment) were used to determine DNA damage. DNA damage was detected by fragmented DNA that caused comet formation by migration from the nuclei of bone marrow cells. But nuclei without comets were considered normal[15].

Statistical Analysis

The Graphpad PRISM (Graphpad Software Inc., Version 8.0d) program was used for statistical analysis. In the comparisons between the groups, one-way analysis of variance was used for the variables with normal distribution, and multiple comparisons were made with the Bonferroni test in case the distribution was different. While comparisons were made between the groups in the variables that did not show normal distribution with the Kruskal-Wallis Analysis, in case of difference, multiple comparisons were made with the Mann Whitney U test. The statistical significance was accepted at a level of p-value less than 0.05.

RESULTS

Micronucleus Frequency in Control and Experimental Groups

A total of 1000 PCEs were counted in the bone marrow preparations of each animal for the MN test (Figure 1). The MNPCE number was determined in these 1000 counted cell. A total of 7,000 cells were scored per group (1000 PCE per rat analyzed; seven rats/group). In addition, a total of 2000 erythrocytes were counted from each rat sample and PCE/NCE ratios were calculated. The MNPCE and PCE/NCE ratios obtained from the control and experimental groups are shown in Table I.

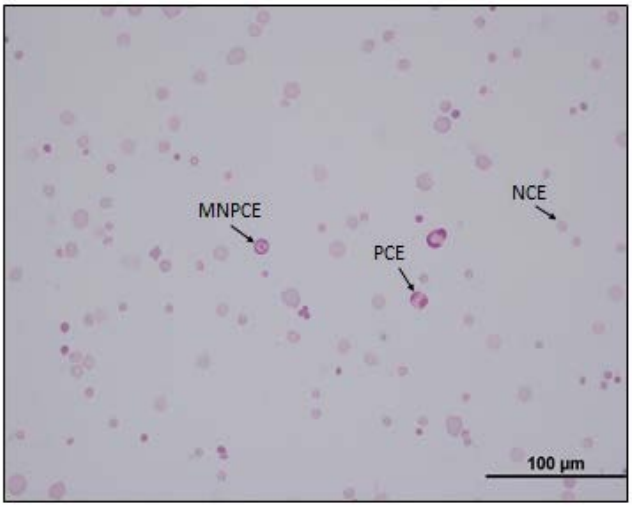

Figure 1. Normal polychromatic (PCE) and normochromatic (NCE) erythrocytes and Micronucleus polychromatic erythrocytes (MNPCE) in rat bone marrow (Giemsa staining X40, Olympus BX51).

Table I: Frequencies of MNPCE and the ratio of (PCE/NCE) in rat bone marrow.

	Control Median (Q1-Q3)	LPS Median (Q1–Q3)	GL Median (Q1–Q3)	LPS+GL Median (Q1–Q3)
Ratio of (PCE/NCE)	0.1810 (0.1190-0.2060)	0.3055 (0.205-0.3543)	0.1330 (0.1080-0.2940)	0.1780 (0.1530-0.2780)
%MNPCE	0.40(0.050-1.35)	1.3(0.9250-1.6)	0.4(0.2-1.3)	1(0.8-1.2)

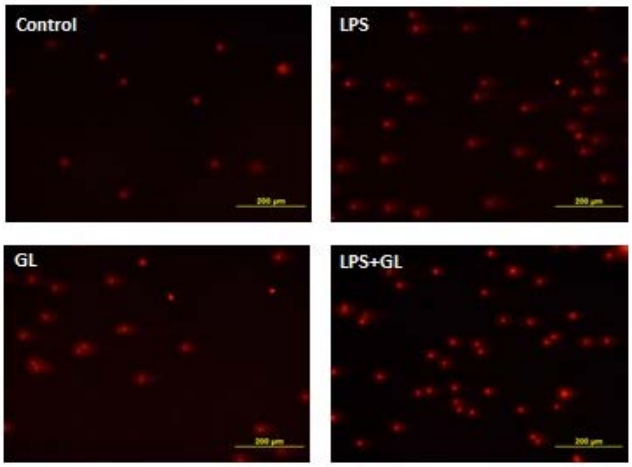

Figure 2. Comet images of control and experimental groups (Ethidium bromide staining x400, Olympus BX51).

Determination of DNA Damage

All parameters necessary to calculate the amount of DNA damage were evaluated (Figure 2). It resulted in cellular DNA damage in the bone marrow cells of the experimental groups and an increase in the comet parameters of the other groups was observed compared to the control group.

The comet parameters (except head DNA) of experimental groups were significant increased according to control group (Table II). When we look at the L Head value, there was a significant difference between the experimental groups and the control group ($p<0.001$), but there was no significant difference between them. At the same time, no significant difference was observed between the control group and the LPS+GL group in head DNA ($p>0.05$). However, there was significance between the experimental groups in head DNA ($p<0.001$). L Tail, Tail DNA, OTM values were significantly different both compared to the control and among themselves ($p<0.001$). While there was a significant difference between the LPS+GL group and the LPS and GL groups in the TM value and L comet value ($p<0.001$), no difference was observed between the LPS and GL groups ($p>0.05$).

Table II. Statistical results of comet parameters in control and experimental groups.

	Control Median (Q1-Q3)	LPS Median (Q1–Q3)	GL Median (Q1–Q3)	LPS+GL Median (Q1–Q3)
L Head	132(121-141.5)[a]	158(139-167.5)[b]	153(139-168)[b]	155(141-167)[b]
L Tail	28(20.75-35)[a]	124.5(107.5-137.3)[b]	99(90.75-114.3)[c]	77.5(69.5-85.5)[d]
L Comet	161(146-173.3)[a]	270(233.3-289.3)[b]	242(220.3-276)[b]	220(186.3-243.5)[c]
Head DNA	95(78-96)[a]	76.5(75-78)[b]	81(81-82)[c]	87(84.75-88.25)[a]
Tail DNA	4(3-5)[a]	23.5(22-25)[b]	19(18-19)[c]	13(11.75-15.25)[d]
TM	1(1-2)[a]	22.5(16.75-27.25)[b]	17.5(15-19)[b]	10.5(8-13)[c]
OTM	3(2-3.25)[a]	24.5(19.75-26.25)[b]	17(14.75-20)[c]	12(10-14)[d]

The same letters on the same line indicate similarity between groups and different letters indicate difference between groups (L Head: Length Head; L Tail: Length Tail; L Comet: Length Comet; Head DNA: % DNA in head; Tail DNA: % DNA in tail; TM: Tail Moment; OTM: OliveTail Moment).

DISCUSSION

Genotoxic agents are substances that cause adverse effects on cell DNA, in other words, degrade genetic material. These negative effects are passed on to future generations. Human beings are inevitably exposed to increasing amounts of genotoxic substances every day. It is important to examine the effects of such chemical compounds in detail on rodents such as rats or mice, or on rabbits[16]. In recent years, medicinal mushroom cultivation has been increasing in Turkey, as in the rest of the world, and Ganoderma lucidum is one of them. Ganoderma lucidum has become a very popular medicinal mushroom in recent years due to its anticarcinogenic and immune system-enhancing effects and its protective effect against infections in the human body[17].

Micronuclei consist of acentric fragment and/or a complete chromosome that is not pulled to the poles in mitosis. Nuclear membrane forms around the chromosomes and fragments that have been separated in telophase, and thus micronuclei, which are smaller than the main nucleus, are formed. This method can only be used in cells capable of dividing[18]. The micronucleus test is a very valuable tool for determining the genotoxic and carcinogenic potentials, as well as the safety, of a wide range of chemicals that we are exposed to on a daily basis[19].

If bone marrow is to be studied, erythrocytes are used in the micronucleus test. In the erythropoiesis stage, after the last mitosis, erythroblasts in the bone marrow lose their nuclei while transforming into polychromatic erythrocytes (PCE), and the chromosomal damage that occurs during this period causes MN formation in the cytoplasm. An increase in the number of immature (polychromatic) erythrocytes (MNPCE) containing MN is an indicator of chromosomal damage or cytogenetic damage resulting from anaphase delay. The fact that the PCE/NCE ratio in chemically treated animals was much lower than in the control group suggests that the administered chemical penetrates the bone marrow and has a deleterious effect on nucleated cell development and maturation[11]. As a result of the studies, it was found that large micronuclei cause the angiogenic effect caused by a chromosome loss, while small micronuclei cause loss of genetic material[20].

Endotoxins have a wide range of pathologic consequences. When an organism is exposed to LPS, it may have a systemic inflammatory response, resulting in a variety of pathophysiological consequences such as endotoxin shock, tissue destruction, and death[21]. When we look at the literature, although there are enough studies that reveal the biological and biochemical properties of Ganoderma lucidum, conflicting results have been revealed regarding its protection against DNA damage. In a previous study, it was stated that Ganoderma lucidum did not significantly reduce the rate of MN increased by mitomycin-C, that is, it did not show an antigenotoxic effect[22]. Considering the benefits of Ganoderma extracts, it has been shown that it has a protective effect on DNA, especially against UV radiation-induced breaks by preserving the structural integrity of the DNA helix[23]. In studies conducted with the MN method and plasmid DNA method in mouse bone marrow, it was determined that the MN formation triggered by gamma radiation was reduced by Ganoderma lucidum polysaccharides in a dose-dependent manner[24]. In this study, it was seen that the results obtained from the LPS and LPS+GL groups increased the micronucleus formation compared to the control group. According to our findings, we can say that LPS can cause chromosomal damage in the cell. We can state that when Ganoderma lucidum is applied together with LPS, not alone, it reduces the frequency of MN in polychromatic erythrocytes and this is an indicator of antigenotoxic activity.

At the same time, it can be said that the damage caused by LPS reduces the damage by activating the Ganoderma lucidum repair mechanisms.

Chemicals' in vitro cytotoxic effects have also been linked to the creation of DNA strand fractures, according to some researchers. The alkylating capabilities of the compounds generated chromosomal anomalies, and the alkylating agents caused DNA damage[25]. In a study, it was stated that Ganoderma polysaccharides given after gamma radiation decreased the Comet values to normal levels and increased the repair mechanism of polysaccharides positively[26]. Smina et al. The protective effect of different concentrations of triterpenes isolated from Ganoderma lucidum against damage in lymphocytes exposed to γ-radiation was evaluated by the comet. As a result, in DNA damage caused by irradiation, triterpene application has been shown to effectively reduce the damage[27]. Chiu et al. showed that DNA breaks increased with EMS uptake as a result of the in vivo comet test used to determine genotoxicity in mouse lymphocytes injected with ethyl methanesulfanate (EMS). It was revealed that there was no significant difference between the control group and the group given only Ganoderma extract. In addition, no protective effect was observed against the genotoxicity of EMS[28]. Damaged DNA fragments travel at various speeds in the electric field because they have varied molecular weights and electrical charges as a result of the damage, and the resulting DNA migration images were assessed to identify the level of LPS damage in this work. In terms of LPS-induced DNA damage, it was observed that it was higher than the control group when other parameters except Head DNA were compared. However, it was observed that Ganoderma lucidum statistically protected the damage when applied together against the damage caused by LPS. However, DNA damage was also observed in the group administered Ganoderma lucidum alone.

Today, different cytotoxic and genotoxic substances cause structural alterations in DNA, DNA chain breakage, and cancer in humans and other living things. By interacting with chemical, physical, and biological factors, changes in DNA structure can promote mutation, apoptosis, and cancer formation[29]. It has been determined that Ganoderma lucidum has a genoprotective effect at low concentrations, but damages DNA at high concentrations. It has been determined that non-damaging concentrations of Ganoderma lucidum have a protective effect against oxidative components that cause harmful effects in cells. The protective effect of this fungus at low concentrations may explain its curative effect[30]. Chemical agents, drugs, toxins, UV light and ionizing radiations play an important role in DNA damage. Single or double chain breaks that occur as a result of damage and are not repaired can cause cell death. Simple breaks in a single strand are immediately repaired by the enzyme DNA ligase. Repair of double-strand breaks is achieved by two major mechanisms: homologous recombination (HR) and non-homologous end joining[29]. When all criteria are evaluated together in terms of comet test, it cannot be said that it is a genotoxic or clastogenic agent. But we can say that it reverses the said genotoxic damage. It is thought that Ganoderma lucidum and LPS cause single and/or double strand breaks on the DNA strand, but these damages are repaired by DNA repair mechanisms, so there is no statistical difference in the number of MN, which is a biomarker of chromosome breaks and/or complete chromosome loss. These results need to be supported by further studies.

Ethics Committee Approval: The approval of the Erciyes University Animal Experiments Local Ethics Committee for the conduct of this

study was obtained with the decision numbered (decision no:19/159, date: 11.09.2019).

Conflict of Interest: The authors declared no conflicts of interest.

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REFERENCES

1.Cimen B, Cimen L, Cetin I, Cetin A. Alpha-lipoic acid alleviates lipopolysaccharide-induced liver damage in rats via antioxidant effect. Dicle Med J. 2019; 46: 125-132.

2. Coskun A, Sen I. Acute phase response and clinical changes in calves with lipopolysaccharide induced endotoxemia. Eurasian J Vet Sci. 2012; 28: 21-26.

3. Aly HA, El-Beshbishy HA, Banjar ZM. Mitochondrial dysfunction induced impairment of spermatogenesis in LPS-treated rats: modulatory role of lycopene. Eur J Pharmacol. 2012; 677: 31-8.

4. Yavaş MC, Akpolat V, Deveci E, et al. Determining the effect of an electromagnetic field generated by a high voltage power line on rat spermatogonia cells. Dicle Med J. 2018: 45: 447-461.

5. Lee S, Park S, Oh JW, Yang CH. Natural inhibitors for protein prenyltransferase. Planta Med. 1998; 64: 303-308.

6. Boh B, Berovic M, Zhang J, Zhi-Bin L. Ganoderma lucidum and its pharmaceutically active compounds. Biotechnol Annu Rev. 2007; 13: 265-301.

7. Dusinska M, Runden-Pran E, Carreira SC, Saunders M. "Critical evaluation of toxicity tests": Adverse effects of engineered nanomaterials: exposure, toxicology and impact on human health. Elsevier. 2012; 63-84.

8. Şekeroğlu V, Atlı-Şekeroğlu Z. Genotoksik hasarın belirlenmesinde mikronükleus testi. Türk Hijyen ve Deneysel Biyoloji Dergisi. 2011; 68: 241-252.

9. Donmez-Altuntas H, Bitgen N. Evaluation of the genotoxicity and cytotoxicity in the general population in Turkey by use of the cytokinesis-block micronucleus cytome assay. Mutat Res. 2012; 748: 1-7.

10. Coskun M, Cayir A, Coskun M, Tok H. Evaluation of background DNA damage in a Turkish population measured by means of the cytokinesis-block micronucleus cytome assay. Mutat Res. 2013; 757: 23-7.

11. Lambert IB, Singer TM, Boucher SE, Douglas GR. Detailed review of transgenic rodent mutation assays. Mutat Res. 2005; 590: 1-280.

12. Vasquez MZ. Combining the in vivo comet and micronucleus assays: a practical approach to genotoxicity testing and data interpretation. Mutagenesis. 2010; 25: 187-99.

13. Pu X, Wang Z, Klaunig JE. Alkaline comet assay for assessing DNA damage in individual cells. Curr Protoc Toxicol. 2015: 65; 3-12.

14. Iraz M, Iraz M, Eşrefoğlu M, Aydın MŞ. Protective effect of β-glucan on acute lung injury induced by lipopolysaccharide in rats. Turk J Med Sci. 2015; 45: 261-7.

15. Akdağ MZ, Daşdağ S, Cantürk F, Karabulut D, Caner Y, Adalier N. Does prolonged radiofrequency radiation emitted from Wi-Fi devices induce DNA damage in various tissues of rats? J Chem Neuroanat. 2016; 75: 116-122.

16. Özparlak H. Tavuk embriyolarının embriyotoksisite ve teratojenite testlerinde kullanımı. Selçuk Üniversitesi Fen Fakültesi Fen Dergisi. 2015; 40: 13- 22.

17. Üstün O. Makrofungusların besin değeri ve biyolojik etkileri. Turk Hij Den Biyol Derg. 2011; 68: 223-240.

18. Fenech M. The in vitro micronucleus technique. Mutat Res. 2000; 455: 81-95.

19. Demirel C, Kilciksiz S, Ay OA, Gurgul S, Ay ME, Erdal N. Effect of N-acetylcysteine on radiation-induced genotoxicity and cytotoxicity in rat bone marrow. J Radiat Res. 2009; 50: 43-50.

20. Angus DC, Pereira CAP, Silva E. Epidemiology of severe sepsis around the world. Endocrine, Metabolic & Immune Disorders - Drug Targets. 2006; 6: 7-16.

21. Ogıkubo Y, Norimatsu M, Noda K, et al. Evaluation of the bacterial endotoxin test for quantification of endotoxin contamination of porcine vaccines. Biologicals. 2004; 32: 88-93.

22. Çayır U. Ganoderma lucidum'un İnsan Periferal Lenfositlerinde Genotoksik Ve Mitomisin-C'ye Karşı Antigenotoksik Etkileri. Gazi Üniversitesi, Fen Bilimleri Enst. 2012.

23. Shi Yl, James AE, Benzie IFF, Buswell JA, Mushroom-derived preparations in the prevention of H2O2-induced oxidative damage to cellular DNA. Teratog Carcinog Mutagen. 2002; 22: 103-11.

24. Pillai TG, Nair CKK, Janardhanan KK. Polysaccharides isolated from Ganoderma lucidum occurring in Southern parts of India, protects radiation induced damages both in vitro and in vivo. Environ Toxicol Pharmacol. 2008; 26: 80-85.

25. İlce Z, Bekdemir FO, Pandır D. Acute toxic effect of lipopolysaccharides to blood tissue in rats and responses to vitamin E and sodium selenite. J Food Biochem. 2019; 43: e13060.

26. Pillai TG, NairC. Janardhanan K. Enhancement of repair of radiation induced DNA strand breaks in human cells by Ganoderma mushroom polysaccharides. Food Chemistry. 2010; 119: 1040-1043.

27. Smina TP, De S, Devasagayam TPA, Adhikari S, Janardhanan K. Ganoderma lucidum total triterpenes prevent radiation-induced DNA damage and apoptosis in splenic lymphocytes in vitro. Mutat Res. 2011; 726: 188-94.

28. Chiu SW, Wang ZM, Leung TM, Moore D.Nutritional value of Ganoderma extract and assessment of its genotoxicity and anti-genotoxicity using comet assays of mouse lymphocytes. Food Chem Toxicol. 2000; 38: 173-178.

29. Araldi RP, Correea de Melo T, Mendes TB, et al. Using the Comet and micronucleus assays for genotoxicity studies: A review. Biomed Pharmacother. 2015; 72: 74-82.

30. Wachtel-Galor S, Choi SW, Benzie IFF. Effect of Ganoderma lucidum on human DNA is dose dependent and mediated by hydrogen peroxide. Redox Rep. 2005; 10: 145-149.