Annals of Clinical and Analytical Medicine
Original Research

Genotoxicity of pyrethrin misuse as chamomile substitute

Pyrethrin/chamomile genotoxicity

Merve M. Cıceklıyurt[1], Gulsum Akkus[2], Begum Dermencı Tufan[3], Hande İpek[3], Sibel Oymak Yalçın[4]
[1] Department of Medical Biolog, Faculty of Medicine
[2] Department of Biology, Faculty of Graduate Studies
[3] Department of Medical System Biology, Faculty of Graduate Studies
[4] Department of Public Health, Faculty of Medicine, Canakkale Onsekiz Mart University, Canakkale, Turkey

Abstract
Aim: Chrysanthemum cinerariaefolium is a white-yellow, daisy-like plant known for more than one hundred fifty years of insecticide property. Although active ingredients of Chrysanthemum cinerariaefolium, pyrethrin is less toxic than organophosphate insecticides, adverse effects on immune system have been demonstrated in numerous animal studies. In our study, the genotoxic potential of accidental consumption (by mixing or unintentional causes) of Chrysanthemum cinerariaefolium instead of chamomile (Matricaria recutita) is investigated.
Material and Methods: Lymphocyte isolation was performed from five male, five female donors from peripheral blood samples. Cytotoxic and genotoxic effects of pyrethrin were investigated in human peripheral lymphocyte cultures with chromosome abnormalities (CA). Micronucleus (MN), mitotic index (MI), and nucleus division index (NDI) were calculated. Cultures were treated with mixed doses of pyrethrin and chamomile in different ratios.
Results: All doses compared with negative control MN, binucleate, tetranucleate, and MI were significantly increased. In the MN assay, micronucleus formation has been increased due to the gradual increase of pyrethrin/chamomile concentration. In chromosome anomaly test, results differed compared with negative and positive control, and in 24 and 48-hour applications of 1/1 mixed pyrethrin and chamomile samples were founded genotoxically.
Discussion: As a result, we have observed pyrethrin has dose-related toxicity increase within the combination. We conclude that the effect of long-term accidental consumption trigger MN, binucleate, tetranucleate formation together with chromosome and chromatin type aberrations.

Keywords
Toxicology, Genotoxicity, Chromosome Aberration, Micronucleus

DOI: 10.4328/ACAM.22021 Received: 2023-10-25 Accepted: 2023-12-11 Published Online: 2024-06-25 Printed: 2024-08-01 Ann Clin Anal Med 2024;15(8):531-535
Corresponding Author: Sibel Oymak Yalçin, Department of Public Health, Faculty of Medicine, Canakkale Onsekiz Mart University, 17100, Canakkale, Turkey.
E-mail: cevizcisibel@gmail.com P: +90 554 866 79 32
Corresponding Author ORCID ID: https://orcid.org/0000-0001-7979-8892
This study was approved by the Ethics Committee of Çanakkale Onsekiz Mart University (Date: 2018-07-11, No: 2018/13-03)

Introduction

Chrysanthemum cinerariaefolium (CC) and Matricaria recutita (MR) are both members of the Asteraceae family. Matricaria recutita, known as chamomile, is widely used in traditional medicine recognized for its antimicrobial, antispasmodic, anxiolytic, immunomodulatory, and sedative purposes via therapeutically active compounds as sesquiterpenes, flavonoids, coumarins, and polyacetylenes, etc. Chrysanthemum, extensively used as a natural insecticide, attracts attention by its morphological similarity to Matricaria recutita [1]. In contrast, Chrysanthemum cinerariaefolium stimulates the nervous systems, so it triggers hyperexcitability. The resemblance of these plants to each other may lead to unintentional accidental consumption of adulteration by commercial. Notably lacking data on this medical condition are data on the genotoxic effects of these two species in the presence of a mixture of CC and MR at different rates on human use.

The bioactive ingredient of Chrysanthemum cinerariaefolium is pyrethrin, a mixture of insecticidal components named pyrethrum [2-3]. Pyrethrin poisoning occurs due to inadvertently exposure to pyrethrin or usage of products that consist of pyrethrin in agricultural or living areas. According to reports, inadvertent exposure cases constituted nearly all cases (95% of reported cases), and %93 of these incidents have been reported to occur around the home [4].

The primary mechanism of action of pyrethrin has shown the prolongation of voltage-gated sodium channels' closing time after sodium infusion in depolarization phase of action potential in nerve cells [2]. As a result, it causes neurotoxicity with hyperexcitability. The case reports indicate that exposure to pyrethrin causes pulmonary adverse effects and painful paresthesia. Paresthesia commonly occurs with dermal exposure to pyrethrin [3,5]. Muscle cells are also affected by pyrethroids' toxicological properties by disrupting sodium and chloride channels and cause muscle fasciculations [6].

Due to the resemblance between these two plants, the research's objective is to investigate which results will occur when intentional or unintentional reasons adulterate these two plants. The study aims to compare genotoxic effect of pyrethrin in case of mixing with medicinal plant Matricaria recutita (Chamomile) with different proportions. Our study is the first one that examines the genotoxic effects of either Chrysanthemum cinerariaefolium alone or when mixed with Matricaria recutita extract on human peripheral blood cells.

Material and Methods

Participants

The study population consisted of 10 healthy volunteers (20-30 years of age) with no exposure to genotoxic agents. All volunteers were informed about the study before. A well-structured questionnaire form was filled through personal interviews. None of the participants use cigarettes, alcohol, or drugs and are non-exposed to DNA-damaging cytostatic drugs or radiation within one year. All this information was obtained from detailed questionnaire and confirmed twice.

Method

For this study, Matricaria recutita flowers have been bought from the local market as test material and dried. Plant flowers (20 g) were extracted with 200 ml of water in an ultrasonic water bath for 24 hours. After the extraction process, obtained solution was distilled. Then the rest of the water was evaporated [7]. The stock solution was prepared by diluting extract (100 mg) by adding 20 ml of water. After all, the stock solution was stored in colored vials at 4°C for further experiments. Chrysanthemum cinerariaefolium (CC) extract and Matricaria recutita (MR) extract were mixed in 1/10, 1/5, 1/2, and 1/1 concentrations to examine genotoxic or protective effects on human lymphocytes. Pyrethrin (Sigma-Aldrich Lot no: BCBW1931) was used as Chrysanthemum cinerariaefolium extract. The investigation consists of 6 study groups [1/10 study group 1CC:9 MR (V/V), 1/5 study group 1CC:4 MR (V/V), 1/2 study group 1CC:1 MR (V/V), 1/1 study group 1CC without MR-only pyrethrin, NC as Negative Control, PC as Positive Control].

Lymphocyte Cultures and Dose Selection

Peripheral blood samples were collected from the healthy individuals in 5 CC sterile heparinized tubes. Heparinized whole blood (0.2 ml) was added in the cell culture medium mixture containing 2,5 ml Chromosome B medium (Merck Millipore) and then incubated at 37 °C. The CC and MR were mixed in 1/10, 1/5, 1/2 and 1/1 concentrations. The positive (MMC- 0.2 mg/ml) (PC) and negative (dH2O) control (NC) and all 5 different doses were added separately to culture after 24 h incubation [8].

Micronucleus (MN) Assay

Cytochalasin B (Sigma-Aldrich, CAS No: 14930–96–2) (6 mg/mL) was added to each culture 44 h after cell culture initiation to arrest cytokinesis. The cultures were incubated for a total of 72 h at 37 °C. The cells were fixed at the end of the incubation period. Cells were first treated with cold potassium chloride (0.075 M) as hypotonic solution. The culture tubes were centrifuged at 1000 rpm for 5 min; then, supernatant was discarded. Pellet was resuspended and fixed in methanol–acetic acid (3:1, v/v) three times. Homogenized pellet dropped in slides and after fixing, the slides were stained with Giemsa (5%).

Chromosome Aberration Analyses

The procedure of Evans (1984) and Perry & Thompson (1984) were followed with minor modifications for chromosome aberration (CA) tests [9,10].

Metaphase Scoring

A total of 250 metaphases scored for CA tests according to the International System for Human Cytogenetic Nomenclature. Structural chromosomal aberrations (SCA), including both chromatid and chromosome type aberrations, were evaluated. Chromatid-type exchanges were considered in the gap, break, complex formation, and total chromatid-type exchanges. Besides, chromosome-type abnormalities were assessed in chromosome gap, break, dicentric chromosome, ring chromosome, acentric fragment, marker chromosome, and total chromosome-type exchanges.

Slide Analysis

The microscopic evaluation of slides was done under a light microscope (Euromex-Zeiss, Germany) at × 1000 magnification [8]. Mitotic index (MI) was calculated as a ratio of cells undergoing mitosis to the total number of cells. Binucleated cells were evaluated [8, 11]. A total of 1000 cells from each sample were analyzed from each individual (10000 cells).

Statistical Analysis

Obtained data analyzed with SPSS V.17.0 (SPSS, Inc. Chicago, IL, USA) pack program. The analysis of variance (ANOVA) followed by a One-Way ANOVA test was used in this study.

Ethical Approval

Written informed consent has been obtained from each subject before the sampling, and the whole study was conducted under Declaration of Helsinki, World Medical Association, 2013. This study was approved by the Ethics Committee of Çanakkale Onsekiz Mart University (Date: 2018-07-11, No: 2018/13-03).

Results

In this study, genotoxic potential of Matricaria recutita and Chrysanthemum cinerariaefolium extracts alone and their mixture in different proportions were investigated in human lymphocyte cultures.

The CC/MR mix was compared to control for MI, MN, binucleus, and tetranucleus (Table 1 and Figure 1).

Compared to NC, pyrethrin only dose was toxic, and this toxicity was as potent as the PC. 1/9 group is not statistically different from the NC. Depending on the dose increase, an increase in toxicity was observed in 1 CC / 4 MR and 1 CC / 1 MR groups in the formation of MN.

However, this increase is not significant. While the genotoxicity potential of 1/10 group is low, it starts to trigger the formation of MN at 1/5 dose.

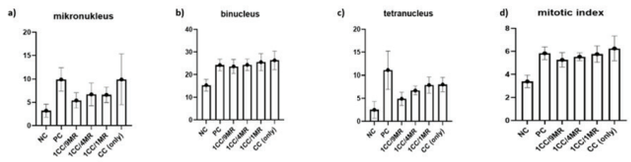

Figure 1. Comparations of different dose compositions and controls

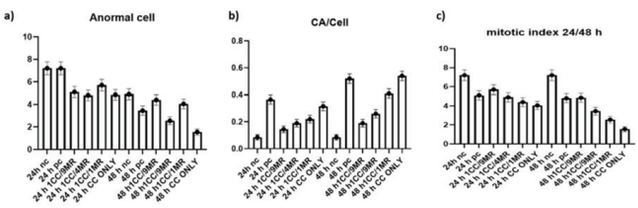

Figure 2. Comparations of different dose compositions and controls in terms of Abnormal cells (a), Chromosomal Aberrations per cell (b) and Mitotic Index (c)

It has been observed that all concentrations trigger binucleus formation, and all quantities are as toxic as PC group. It was observed that no combination generates tetranucleus shape as much as PC; however, 1/5 group and higher concentrations significantly increased tetranucleus formation compared to NC. There is a significant increase in dose-dependent tetranucleus formation. While (1/10 and 1/5 groups) and (1/1 and CC only) concentrations did not differ in TN formation among themselves, there was a statistically significant difference between them.

The percentage of MI used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression, and MI data are given in Figure 2.

Our study has proven that both exposure time and increasing rate of pyrethrin trigger both abnormality frequency and amount of abnormal cells. Cause of MI decrease in increased pyrethrum exposure ratio group can be explained by mechanism of genotoxicity inhibits cell cycle due to chromosomal aberrations. It seems that increasing rate of pyrethrin triggers forming of chromatid and chromosome type anomalies clearly. Thus, MI decreases because abnormal chromosomes or chromatins block cell cycle due to mitotic spindle failure to attack chromosome and move towards poles.

Discussion

Extracts of plants are generally regarded as safe by public; in contrast, dose of extracts or consumption duration is an essential parameter for safety. Polyphenolic compounds of plants are clinically essential to prevent many diseases and generally offer to use as a food additive to prevent disease [12, 13].

Pyrethroids change gating properties by causing delayed closing of voltage-sensitive channels both in mammalian and invertebrate cells. The hazardous effect of the molecule comes from poses a potential risk for malignancies [14]. Acute pyrethroid poisoning was reported in 573 cases in 22 papers between 1983-1988 in Chinese medical literature [2]. Other reports show that, of 37.397 pyrethroids exposures, 1656 resulted in moderate effects, and 3 of them died [2]. First report of pyrethrin-associated fatality is that allergic reactions cause irreversible bronchospasm [15]. Growing evidence indicates that pyrethrin or its synthetic forms cause not only allergic and neurologic problems together with life-threatening conditions together with fatality.

Similarities between Chrysanthemum cinerariaefolium and

Table 1. Summary of genotoxicity and cytotoxicity of pyrethrin in the cultured lymphocytes

Ratio of Pyrethrin/ Chamomile	Micronucleus (mean ± SD)	Binucletaed (mean ± SD)	Tetranucleate (mean ± SD)	Mitotic Index (mean ± SD)
NC (dH2O)	3,20±1,40^{a}	15,40±2,63^{a}	2,5±1,84^{a}	3,40±0,53^{a}
PC (MMC (0.5))	9,90±2,47^{b}	24,20±2,62^{b}	11,1±4,12^{d}	5,83±0,56^{bc}
Pyrethrin Only	9,90±5,40^{b}	26,30±4,11^{b}	8,00±1,56^{c}	6,25±1,08^{c}
1CC:1MR	6,60±1,65^{ab}	25,5±3,78^{b}	7,90±1,79^{c}	5,76±0,72^{bc}
1CC:4MR	6,70±2,45^{ab}	24,30±2,54^{b}	6,70±0,95^{bc}	5,53±0,35^{bc}
1CC:9MR	5,40±1,65^{a}	23,6±3,13^{b}	4,90±1,45^{ab}	5,26±0,63^{b}
ONEWAY ANOVA; TUKEY %5				
	8,391 ***	15,307***	17,870***	21,821***

Abbreviations: SD: MMC: Mitomycin C (positive control), Standard deviation, Same value point difference 5% level, * means 5% degree is important, **; 1% is highly significant and ***; ‰ 1 degree is important

Matricaria recutita are a significant risk factor for poisoning, but there is no record if Matricaria recutita accidentally adulterated with pyrethrin.

Nowadays, in vitro MN test in human lymphocytes is widely used in biomonitoring studies to detect clastogens and aneuploidogens [16]. Micronuclei can originate from central fragments (chromosome fragments without centromeres) that cannot migrate with rest of chromosomes during anaphase in-cell division or from complete chromosomes [8]. Percentage of MI or metaphases among harvested fixed lymphocytes requires addition of colchicine to stop progression of cells from metaphase to anaphase and provides enough metaphases for cytogenetic analysis. MI assay is used to characterize proliferating cells and identify compounds that inhibit or induce mitotic progression [11]. Results of present investigation showed that when 24-hour acute and 48-hour applications were compared, it was observed that MI value decreased significantly at all doses. This situation occurs due to genotoxic effect of applied extracts in which affecting microfilament structure (spindle and anaphase formation) during cell division. When 1/10 group was applied for 24 hours, MI value was not affected much. Still, statistically significant increase was observed in number of abnormal cells, and MI rate decreased significantly in 48 hours of application.

Among chromosome aberrations, values such as chromosome and chromatid breakage, displacement, gap formation, and fragments, as well as endoreduplication and polyploidy, were also examined. Although obtained results showed apparent differences compared with negative and positive control, CC and MR samples mixed in 1:1 ratio in 24- and 48-hours applications were evaluated as genotoxic when statistically assessed.

In comparison MMC substance as PC, which is known to be toxic in human lymphocyte culture, with CC and MR mixture doses, it was determined that mixture samples showed lower amounts of MN and binucleate, tetranucleate, and MI levels. However, this micronucleus, binucleate, tetranucleate amounts, and MI levels are considered toxic values in literature [17, 18]. In healthy individuals, MN amount is found in specific ratios. Negative control data confirmed that selected individuals were healthy. Azab et al. have been shown dose-dependent increase in SCE and mitotic and proliferative index [19]. Our results are also similar to Azab et al. Also, we have evaluated mixture of CC and MR in different proportions. As a result, we have also observed pyrethrin has dose-related toxicity increase within the combination.

Conclusion

We conclude that the effect of long-term accidental consumption trigger MN, binucleate, tetranucleate formation together with chromosome and chromatin type aberrations. Thus, public health experts must warn people, and people must be aware of the toxic effects of misuse. Finch et al. has been previously shown that a high dose of pyrethrin induces cancer in thyroid gland and Price et al. have reported that pyrethrin trigger liver tumors [20, 21]. Our data shows that pyrethrin extract are still highly toxic when mixed with chamomile, even in small doses. Mixture of pyrethrin with medicinal plant chamomile trigger cytotoxicity, and we have demonstrated this by decrease in MI together with chromosome and chromatin type abbreviations. Also, chromosome aberrations increase in a dose-dependent manner both in 24th and 48th hours of applications. Aside, our results show that pyrethrin has genotoxic effects and requires treatment due to its DNA damaging effects. As discussed above, in all doses, pyrethrin exposure causes MN, binucleate, and tetranucleate formation, yet this exposure might be tolerable due to DNA repair mechanism. Furthermore, dose-effect relationship between MN and cumulative exposure confirms importance of reevaluating recommended doses using new biomonitoring methods and MN, binucleate, tetranucleate amounts, and MI levels. Moreover, results obtained with benchmark dose approach are promising about use of in risk assessment analysis considering its applicability to large size populations.

It is known that many insecticide substances pose a risk of developmental toxicity in early embryonic period [22]. However, Babeľová et al. examined the embryotoxic potential of pyrethroids and found low embryotoxic potential of pyrethroid insecticides other than commercial products. Embryotoxic potential of commercial products is associated with secondary ingredients [23]. When Baskar and Murthy (2018) examined the effects of pyrethroids on neurological system, they found exposure at low doses affected neurological parameters. The potency of action decreased as dose increased [24]. To sum up, we observed that pyrethrin-induced chromosomal aberrations and MN formation are critical risks for public health. The public should be warned of similarity of the genera Matricaria recutita and Chrysanthemum cinerariafolium.

Scientific Responsibility Statement

The authors declare that they are responsible for the article's scientific content including study design, data collection, analysis and interpretation, writing, some of the main line, or all of the preparation and scientific review of the contents and approval of the final version of the article.

Animal and Human Rights Statement

All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compareable ethical standards.

Funding: *Çanakkale Onsekiz Mart University, The Scientific Research Coordination Unit, Project number: TSA-2018-2673.*

Conflict of Interest

The authors declare that there is no conflict of interest.

References

1. Mandiola-Quililongo C, Soto CC, Gática CM, Arriaza MC, Varas G, Fica JE. Evaluation of the Effect of Refined Extracts of Matricaria recutita L and Chrysanthemum coronarium L, Proposed as Bio-Pesticides for Agricultural Use, Determining the Variation of Biomarkers of Oxidative and Hepatotoxic Stress in Acutely Exposed Rats. Agricultural Sciences. 2020;11(12):1143-58.

2. Bradberry SM, Cage SA, Proudfoot AT, Vale JA. Poisoning Due to Pyrethroids. Toxicol Rev. 2005;24(2):93-106.

3. Hudson NL, Kasner EJ, Beckman J, Mehler L, Schwartz A, Higgins S, et al. with pyrethrin and pyrethroid exposures 11 states, 2000-2008. Am J Ind Med. 2014;57(1):15-30.

4. Hernández-Moreno D, Soffers AE, Wiratno W, Falke HE, Rietjens IM, Murk AJ. Consumer and farmer safety evaluation of application of botanical pesticides in black pepper crop protection. Food Chem Toxicol. 2013;56(1):483-90.

5. Perkins A, Walters F, Sievert J, Rhodes B, Morrissey B, Karr CJ. Home use of a pyrethroid-containing pesticide and facial paresthesia in a Toddler: A case report. Int J Environ Res Public Health. 2016;13(8):829-38.

6. Ramchandra AM, Chacko B, Victor PJ. Pyrethroid Poisoning. Indian J Crit Care Med. 2019;23(4):267-71.

7. Háznagy-Radnai E, Czigle S, Zupkó I, Falkay G, Máthe I. Comparison of antioxidant activity in enzyme-independent system of six stachys species. Fitoterapia. 2006;77(7–8):521-4.

8. Fenech M, The in vitro micronucleus technique. Mutat Res. 2000;455(1–2):81-95.

9. Evans HJ. Handbook of Mutagenicity test procedures, Amsterdam: Elsevier

Science Publishers; 1984; p. 405-27.
10. Perry PE, Thompson EJ. The Methodology of Sister Chromatid Exchanges. Handbook of mutagenicity test procedures, Amsterdam: Elsevier Science Publishers BV; 1984; p. 495–529.
11. Holland NT, Duramad P, Rothman N, Figgs LW, Blair A, Hubbard A, et al. Micronucleus frequency and proliferation in human lymphocytes after exposure to herbicide 2,4-Dichlorophenoxyacetic acid in vitro and in vivo. Mutat Res - Genet Toxicol Environ Mutagen. 2002;521(1–2):165-78.
12. Ayuob N, Hawuit E, Mohammedsaleh ZM, Shaalan D, Hawasah MMH, Basheikh KAA, et al. L. Cucurbita Pepo modulates contact dermatitis in depressed rats through downregulation of proinflammatory cytokines and upregulation of antioxidant status. Adv Dermatol Allergol. 2022;39(2):286-97.
13. Zhang Z, Li X, Sang S, McClements DJ, Chen L, Long J, et al. Polyphenols as plant-based nutraceuticals: health effects, encapsulation, nano-delivery, and application. Foods. 2022;11(15):2189-206.
14. Puvula J, Maddu N, Gutam N, Parimal A, Raghavendra PB. The role of pyrethroid derivatives in autophagy and apoptosis crosstalk signaling and potential risk for malignancies. Oncotarget. 2022;13:1323-40.
15. Wax PM, Hoffman RS. Fatality associated with inhalation of a pyrethrin shampoo. Clin Tox. 1994;32(4):457-60.
16. Bolognesi C, Holland N. The use of the lymphocyte cytokinesis-block micronucleus assay for monitoring pesticide-exposed populations. Mutat Res Rev Mutat Res. 2016;770 (Pt A):183-203.
17. Kopjar N, Kašuba V, Rožgaj R, Želježić D, Milić M, Ramić S, et al. The genotoxic risk in health care workers occupationally exposed to cytotoxic drugs - A comprehensive evaluation by the SCE assay. J Environ Sci Health A Tox Hazard Subst Environ Eng. 2009;44(5):462-79.
18. Heddle JA, Hite M, Kirkhart B, Mavournin K, MacGregor JT, Newell GW, et al. The induction of micronuclei as a measure of genotoxicity. Mutat Res. 1983;123(1):61-118.
19. Azab M, Khabour OF, Alzoubi KH, Hawamdeh H, Quttina M and Nassar L. Assessment of genotoxicity of pyrethrin in cultured human lymphocytes. Drug Chem Toxicol. 2017;40(3):251–55.
20. Finch JM, Osimitz TG, Gabriel KL, Martin T, Henderson WJ, Capen CC, et al. A mode of action for induction of thyroid gland tumors by pyrethrins in the Rat. Toxicol Appl Pharmacol. 2006;214(3):253–62.
21. Price RJ, Walters DG, Finch JM, Gabriel KL, Capen CC, Osimitz TG, et al. A mode of action for induction of liver tumors by pyrethrins in the rat. Toxicol Appl Pharmacol. 2007;218(2):186–95.
22. Wang W, Ito T, Otsuka S, Nansai H, Abe K, Nakao Y, Ohgane J, Yoneda M, Sone H. Epigenetic effects of insecticides on early differentiation of mouse embryonic stem cells. Toxicol In Vitro. 2021;75:105174-84.
23. Babeľová J, Šefčíková Z, Čikoš Š, Kováříková V, Špirková A, Pisko J, et al. In vitro exposure to pyrethroid-based products disrupts development of mouse preimplantation embryos. Toxicol in Vitro. 2019;57:184-93.
24. Baskar MK, Murthy PB. Acute in vitro neurotoxicity of some pyrethroids using microelectrode arrays. Toxicol in Vitro. 2018;47:165-77.

How to cite this article:
Merve M. Cıceklıyurt, Gulsum Akkus, Begum Dermencı Tufan, Hande İpek, Sibel Oymak Yalçın. *Genotoxicity of pyrethrin misuse as chamomile substitute. Ann Clin Anal Med 2024;15(8):531-535*

This study was approved by the Ethics Committee of Çanakkale Onsekiz Mart University (Date: 2018-07-11, No: 2018/13-03)