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Effect of Bromelain Against Nickel Genotoxication in Rats

Sıçanlarda Nikel Genotoksikasyonuna Karşı Bromelainin Etkisi

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ABSTRACT

In this study, we aimed to investigate the protective role of bromelain against nickel sulfate genotoxication. Twenty-four healthy adult male Sprague Dawley rats with an average weight of 200 ± 20 g were used in the study. These were divided randomly into four groups (n = 6), including one control and three experimental groups: Group 1 (control), Group 2 (nickel sulfate 20 mg/kg, intraperitoneal [IP]), Group 3 (Bromelain 20 mg/kg, oral gavage), Group 4 (nickel sulfate 20 mg/kg IP + bromelain 20 mg/kg oral gavage). After the ten-day experimental period, the animals were euthanized on the 11th day. Femoral bones were taken from animals dissected on day 11, and micronucleus protocol was applied. At the end of the protocol, micronucleated polychromatic erythrocytes (MNPCE) and polychromatic erythrocytes (PCE) were counted. The statistical results of the counted values were determined. The groups were compared only according to MNCPE, the level of which was statistically significant in the group with bromelain than in the group with nickel sulfate alone ($P < .05$). The data obtained suggested that bromelain at the administered dose (20 mg/kg) is not potentially preventive to the genotoxic effects of nickel sulfate at the administered dose (20 mg/kg).

Keywords: Bromelain, genotoxicity, nickel sulfate, rat

ÖZ

Bu çalışmanın amacı, nikel sülfat genotoksikasyonuna bromelainin karşı koruyucu rolünü araştırmaktır. Çalışmada, ortalama 200 ± 20 gr ağırlığında yirmi dört sağlıklı yetişkin erkek Sprague dawley sıçan kullanıldı. Rastgele olacak şekilde dört grup (n = 6) oluşturuldu. Sıçanlar bir kontrol ve üç deney grubu: Grup 1 (Kontrol), Grup 2 (Nikel sülfat 20 mg/kg, ip), Grup 3 (Bromelain 20 mg/kg, oral gavaj), Grup 4 (Nikel sülfat, 20 mg/kg, ip + bromelain, 20 mg/kg, oral gavaj). On günlük deney periyodundan sonra hayvanlar on birinci gün ötenazi edildi. 11. günde ötenazi edilen hayvanlardan femur kemikleri alındı ve mikronükleus protokolü uygulandı. Protokolün sonunda MNPCE (mikronükleuslu polikromatik eritrositler) ve PCE (polikromatik eritrositler) sayıldı. Sayılan değerlerin istatistiksel sonuçları belirlendi. Tüm gruplar sadece MNCPE parametresine göre karşılaştırıldı. MNCPE seviyesi, bromelainli grupta, sadece nikel sülfatlı grubuna kıyasla istatistiksel olarak anlamlıydı ($P < ,05$). Elde edilen veriler, bromelainin 20 mg/kg dozunda uygulanması, 20 mg/kg dozunda uygulanan nikelsülfatin oluşturduğu genotoksik etkilerini potansiyel olarak önleyici olmadığını göstermiştir.

Anahtar Kelimeler: Bromelain, genotoksisite, nikel sülfat, rat.

INTRODUCTION

Excessive or long-term intake of metals found in nature, which are needed in small amounts for the regulation of normal vital functions, gives rise to multiple health problems. Nickel, a carcinogenic, genotoxic, teratogenic, and immunotoxic heavy metal, is an environmental pollutant that is not necessary for vital functions. People are particularly exposed to nickel through nickel-contaminated vegetables, spinach, legumes, nuts, and tobacco containing nickel. It has been stated that there is a high amount of nickel, especially in products containing cocoa powder and baking powder.[1-3] In addition, nickel causes food contamination through hydrogenation of vegetable oils and some processes applied to foods with tools and equipment and environmental pollution through processes using nickel in intensive steel and battery production and releasing it to the atmosphere during mining, smelting, and refining. Humans and animals intake nickel through respiration, food, and skin absorption resulting in tissue and organ damage from nickel accumulation.[4] Nickel binds tightly to bases in DNA and RNA in living organisms; phosphate groups; nucleotides such as ATP and thiamine pyrophosphate; amino acids such as methionine, cysteine, histidine, and pyrroline; proteins; peptides; and phospholipids; and substances such as acetyl coenzyme A, dihydrolipoic acid, pyridoxal and pyridoxamine and blocks various enzymes (such as aspartase, alkaline phosphatase, and ATPase) and makes iodine unavailable to the thyroid gland.[5] Multiple studies have determined that nickel increases lipid peroxidation in various tissues such as blood, liver, muscle, and kidney and causes oxidative stress.[6,7]

In recent years, interest in the use of various antioxidant substances as food supplements has been increasing rapidly to prevent the harmful effects of xenobiotics.[8] Bromelain is a natural pro-

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tein decomposer found in pineapple, which has antimetastatic, immunomodulatory, anti-edema, anti-inflammatory, antithrombotic,[9] antioxidant,[10,11] tissue regeneration enhancing, and pain reliever effects.[12]

Previous studies have reported that bromelain and fresh pineapple juice have DNA protective potential, reduce cytotoxity, and have antigenotoxic and antimutagenic effects.[13-17]

In this study, we aimed to evaluate the effects of bromelain on genotoxicity against intraperitoneal nickel sulfate administration in rats.

MATERIAL AND METHODS

All the experimental protocols in this study were carried out in the Experimental Research Center of Harran University. Ethical approval permission was given by the Harran University Animal Experiments local ethics committee (Date: April 5, 2017, No: 12541).

Animals

In our experiments, adult male rats (n = 24, Sprague-Dawley, 6 weeks old weighing 200 ± 20 g) were obtained from an experimental research unit at Firat University. In classic laboratory conditions (12 h light and 12 h dark, 23 ± 2°C, 60%–65% humidity), the animals were fed with a routine diet. Food and water were provided *ad libitum*.

Experimental Design

A total of 24 Sprague-Dawley rats, six in each group, were used. The study was planned as follows: Group 1 control (normal saline intraperitoneal injection [IP]); Group 2 with nickel sulfate (Acros Organics nickel sulfate heptahydrate, Code: 270552500, Lot: A0371683) 20 mg/kg IP[6,18]; Group 3 with bromelain (Sigma B4882 List 647-014-00-9 Cas No 37189-34-7) 20 mg/kg oral gavage[19,20]; Group 4 with nickel sulfate (20 mg/kg, IP) + bromelain (20 mg/kg oral gavage). After 10 days of administration, tissue samples were taken after euthanasia on the 11th day.

In the study, bone marrow was used for micronucleus detection. The removed femur bone was cut from both ends and transferred to a centrifuge tube containing 3 mL of calf serum with the help of a bone marrow injector. Tubes containing bone marrow samples were centrifuged at 2000 rpm for five minutes, and supernatants were discarded. It was suspended by placing a drop of calf serum on the part remaining in the tube. A drop of sample from it was spread on clean slides. After the spreading process, the slides were air-dried and fixed in methyl alcohol for 10 minutes. Bone marrow preparations were prepared by a method that was first developed by Schmid[21] and adapted to this laboratory and working conditions.

Staining Method

The fixed preparations were first stained with 0.25% May Grunwald dye for five minutes and washed with distilled water. It was then stained with 0.125% May Grunwald dye for five minutes and washed in distilled water. Finally, it was stained with 20% Giemsa dye for 30 minutes, washed, and left to dry. The preparations were examined through an Olympus CX21 light microscope at 1000x magnification, and 2000 polychromatic erythrocytes (PCEs) were counted randomly from each preparation. Among these, the numbers of micronucleated polychromatic erythrocytes (MNPCEs) were determined, and their percentages were calculated.

Statistical Analysis

The Kolmogorov-Smirnov test of normality was used to determine whether the data were suitable for normal distribution. Statistical analysis of the data obtained from the study were performed using the IBM Statistical Package for the Social Sciences version 22 (IBM SPSS Corp., Armonk, NY, USA) statistical program. One-way analysis of variance (ANOVA) was used to determine whether there was a difference between the means of the experimental group or not; and if there was a difference between the means of the experimental groups, the "ANOVA-Duncan" test was applied to the group averages to determine which group or groups showed a difference, and $P < .05$ was considered statistically significant. Results were presented as mean ± standard deviation (X ± SD).

RESULTS

In this study, all the groups were compared in terms of the single parameter, MNPCE. As a result of the analysis, the MNPCE level in the group with bromelain was statistically different from the group with nickel sulfate+bromelain and the group with nickel sulfate ($P < .01$). There was no statistically significant difference between the bromelain group and the negative control group ($P > .05$). Again, the difference between the nickel sulfate group and the nickel sulfate + bromelain group was not statistically significant ($P > .05$) (Table 1, Figure 1). Images of MNPCE and normal PCE and NCEs (x1000) in the bone marrow obtained from nickel sulfate group are shown in Figure 2.

Table 1. Statistical data of groups in terms of MNPCE

Parameters	Groups				
	Bromelain (20 mg/kg)	Nickel sulphate (20 mg/kg) +Bromelain (20 mg/kg)	Nickel sulphate (20 mg/kg)	Negatif control	*P*
MNPCE (Mean ± SD)	6.67 ± 1.21^{a}	19.83 ± 1.47^{b}	20.33 ± 1.03^{b}	6.00 ± 1.41^{a}	*

*$P < .01$: Statistically significant difference, a,b: Values with different letter indicate significant differences. SD, Standart deviation

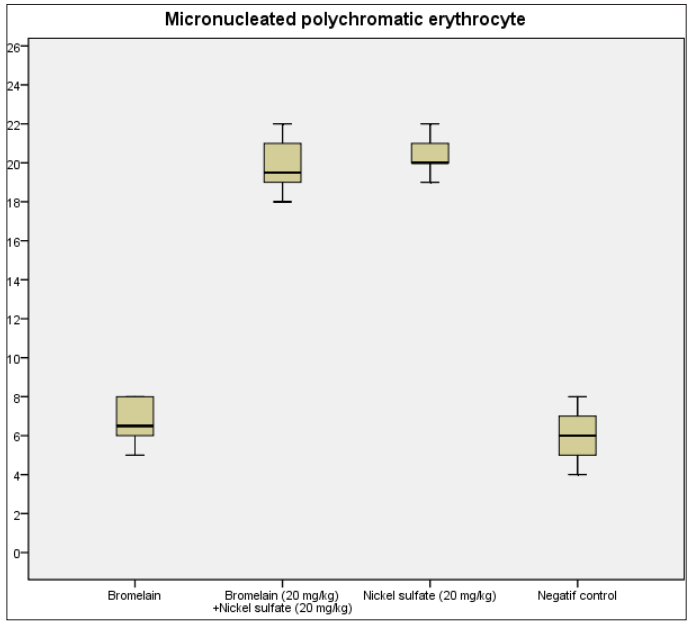

Figure 1. Box graphic showing the numbers of polychromatic erythrocytes with micronucleus based on groups

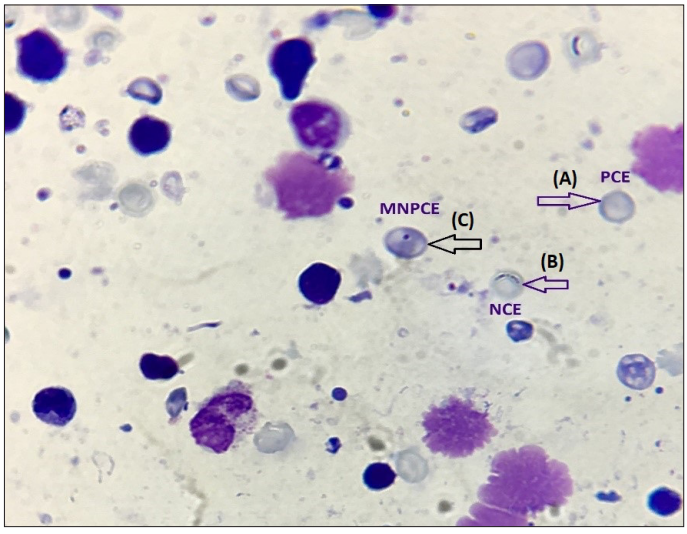

Figure 2. In the bone marrow of rats, images of normal PCE and NCEs, and MNPCE (x1000).
PCE, Polychromatic Erythrocyte; NCE, Normochromatic Erythrocyte; MNPCE, Micronucleated Polychromatic Erythrocyte

DISCUSSION

It is known that nickel compounds damage cell DNA through free radicals. Nickel causes oxidative damage to DNA as well as transduction and replication problems. The water-soluble nature of nickel sulfate increases the carcinogenicity of the substance and thus the mutation potential.[22,23] Many *in vivo* and *in vitro* studies have been conducted on the genotoxic effects of nickel.[24-27] To determine the genotoxicity, comet assay, Salmonella/microsome mutagenicity (AMES) test, sister chromatid exchange assay, chromosome abnormality test, and micronucleus test were performed.[28] According to the literature review, it is noted that bone marrow[29-32] and embryos[5] were used to determine genotoxicity.

In a study, genotoxicity of nickel sulfate hexahydrate was investigated in rats through micronucleus test. In the study, it was determined that nickel sulfate hexahydrate increased the micronucleus frequency in bone marrow cells.[22,27] In another study, it was determined that it increased the kinetochore positive nucleus on human diploid fibroblasts.[25] In this study, we determined that there was a statistically significant difference in MNPCEs in the group with nickel sulfate than that in the other groups ($P < .01$). This result is compatible with other studies which support the idea that nickel sulfate is genotoxic. The increase in the number of PCEs is an indicator of chromosomal damage or cytogenetic damage caused by anaphase delay.[28] PCE/NCE ratio is also expressed as a bone marrow cytotoxicity marker.[29]

Currently, many people support the inclusion of antioxidant substances in diets to protect themselves from the possible harmful effects of environmental pollutants.[33,34] Bromelain, an antioxidant, prevents tumor cell proliferation. It has been determined that bromelain inhibits the proliferation and development of oral cancer cells.[35] It has been determined that bromelain used during sperm freezing to improve sperm quality in goats is not genotoxic.[36]

It has been stated that bromelain can prevent the formation of reactive oxygen groups in the cell. Thus, it can prevent genotoxicity by disrupting the interaction between advanced glycation end products (AGEs) and their receptors (RAGE).[37] In a study examining the genotoxicity of AGE on pig kidney, it was stated that bromelain reduced cell damage and genotoxicity.[38]

In the study by Sen et al.,[39] it was determined that application of 20 mg/kg bromelain protects the sperm DNA fragmentation rate against the damage caused by nickel in the testis, reducing the damage and oxidative stress caused by nickel, and improving sperm quality.

In the literature review, there are various studies in which pineapple and pineapple products were used against the harmful effects of various substances that cause mutagenic effects.

Ikken et al.[40] determined that ethanolic pineapple extract has an antimutagenic effect against the effects of mutagenic nitrosamines. Kurdi[14] stated that pineapple juice had positive results in the micronucleus test against the cytotoxic and genotoxic effects of the antineoplastic drug, ifosfamide, in PCEs of the bone marrow. In another study, it was determined that fresh pineapple juice (0.4 mL/kg/day, oral) was effective in reducing lethal mutation against the side effects of ifosfamide in spermatogenesis stages (early spermatids, primary spermatocytes and spermatozoa, and late spermatid stages) in albino rats.[30] It has been reported that pineapple scavenges reactive oxygen species and protects nucleophilic regions in the DNA.[15] The *in vivo* antitumoral/antileukemic effect of bromelain has been evaluated in different cell lines.[41]

The protective effect of pineapple juice against genotoxicity caused by mutagenic heterocyclic aromatic amines in hamster fibroblasts was determined by the comet assay.[16]

In a study by Sah et al.,[42] the antimutagenic effect of peel powder of pineapple on the yogurt bacteria Lactobacillus was confirmed by the Ames test.

The reason for the difference in the results of *in vivo* and *in vitro* studies may be owing to the pharmacokinetics of the drug in live animals, nutrition, genetic structure, stress, and environmental factors.[12,43] In this study, the antigenotoxic effect of bromelain administered via oral gavage at the same amount was not found in rats administered $NiSO_4$ (20 mg/kg) IP for 10 days. This may be because of the *in vivo* nature of our study.

In the studies conducted by Kurdi,[14,30] it was stated that pineapple juice has an antigenotoxic effect against the cytotoxic and genotoxic effects of ifosfamide in PCEs in the bone marrow and semen formation stages in the micronucleus test. However, because of the presence of different phytochemical structures (such as ascoumaric acid, chlorogenic acid, ferulic acid, and ellagic acid), vitamins (vitamin C, pyridoxine, thiamine, and riboflavin), and minerals (copper and manganese) in pineapple apart from bromelain,[15] these studies do not prove that bromelain alone is effective against genotoxicity.

Drugs and chemicals are primarily dispersed into organs and tissues (brain, heart, kidney, etc.) via blood vessels; and after reaching a certain blood density, they accumulate in fat, bone tissue, nucleic acid, and keratinous structures.[5] Bone tissue has less perfusion than the vital organs.[44] There are many studies conducted on the harmful effects of nickel compounds on the liver, kidney, testes, and sperm causing oxidative stress in living organisms.[6,45-47] In the study by Sen et al.,[39] bromelain showed a protective effect against the harmful effects of $NiSO_4$ on sperm DNA. However, there are different studies in which bromelain does not show a protective effect against genotoxicity. This may be owing

to the different accumulation tendencies of nickel in testicular and bone marrow and the different tissue and perfusion rates of the testes and bone marrow.[44,48]

In conclusion, it was determined that bromelain (20 mg/kg) did not have a protective effect against the genotoxic effects of nickel sulfate.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Harran University (Date: April 5, 2017, No: 12541).

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