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Research Article

Effects of Borax on Inflammation, Haematological Parameters and Total Oxidant-Antioxidant Status in Rats Applied 3–Methylcholanthrene [1]

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Abstract

In this study was investigated effects of borax (BX) on inflammation markers, haematolojical parameters and total oxidant (TOS)-antioxidant status (TAS) in rats applied 3–methylcholanthrene (3-MC). In this research a total of 24 Wistar Albino rats were used. They were divided into 4 groups each containing 6 rats. 1st group was separated as a control group. 3-MC was applied twice a week first 2 weeks 25 mg/kg dose to the 2nd group with i.p. way. BX was given to 3rd group 300mg/L/day dose with drinking water during 150 days. 3-MC was applied twice a week first 2 weeks 25 mg/kg dose with i.p. way and BX were given with drinking water during 150 days to 4th group. At the end of the study blood analysis, tumor necrosis factor alpha (TNF-α) and interleukin 1 beta (IL-1β) levels in 3-MC group; TOS and oxidative stress index (OSI), platelet (PLT) levels in 3-MC and 3-MC+BX groups showed significantly increases when compared to other groups. It was determined that lymphocytes % (LY%) of ever 3 groups were significantly higher; however, neutrophil % (NEU%) were significantly fewer according to control group. Haemoglobin (HGB) and hematocrit (HCT) values of 3-MC+BX groups showed significantly decrease according to other groups (P≤0.05). Mean corpusculer volume (MCV) in 3-MC and 3-MC+BX groups showed significantly decrease when compared to other groups (P≤0.05). As a result, in case of exposure to 3-MC, long-term use of BX with oral ways may not decrease oxidative stress, may changes haematolojical parameters such as, WBC, LY%, NEU%, PLT, HGB, HCT, MCV. However, these changes remain within physiological limits. Even so, in the use of BX should be considered use of iron. Furthermore, BX with the abovementioned dosage may be used to reduce the levels of TNF-α, IL-1β, IL-6 being inflammation and cancer markers.

Keywords: *Borax, Haematology, Inflammation, Interleukin, Rat, Tas, Tos, Tnf-α, 3-MC*

3-Metilkolatren Uygulanan Sıçanlarda Boraksın İnflamasyon, Hematolojik Parametreler ve Total Oksidan-Antioksidan Durumlar Üzerine Etkileri

Özet

Bu çalışmada, 3-metilkolatren (3-MC) uygulanan sıçanlarda boraksın (BX) inflamasyon göstergeleri, hematolojik parametreler ve total oksidan (TOS)-antioksidan durumlar (TAS) üzerine etkileri araştırıldı. Çalışmada toplam 24 Wistar Albino sıçan kullanıldı. Sıçanlar her grupta 6'şar adet olacak şekilde 4 gruba ayrıldı. Birinci Grup kontrol grubu olarak ayrıldı. İkinci gruba 25 mg/kg dozunda haftada iki kez ilk 2 hafta 3-MC i.p. yolla uygulandı. Üçüncü gruba BX 300 mg/L/gün dozunda içme suları ile 150 gün boyunca verildi. Dördüncü gruba 3-MC 25 mg/kg dozunda haftada iki kez ilk 2 hafta i.p. yolla uygulandı ve BX 300 mg/L/gün dozunda içme suları ile 150 gün boyunca verildi. Çalışma sonunda kan analizlerinde, diğer gruplarla karşılaştırıldığında 3-MC grubunda tümör nekrozis faktör alfa (TNF-α) ve interlökin 1 beta (IL-1β); 3-MC ve 3-MC+BX gruplarında ise, TOS, oksidatif stres indeksi (OSI) ve trombosit (PLT) seviyeleri istatistiksel önemde artış gösterdi. Kontrol grubuna göre her 3 gruptaki % lenfosit (%LY) seviyeleri yüksek; fakat % nötrofil (%NEU) seviyeleri önemli düzeyde düşük olduğu belirlendi. 3-MC+BX grubunda hemoglobin (HGB) ve hematokrit (HCT) değerleri diğer gruplara gore önemli bir azalma gösterdi (P≤0.05). 3-MC ve 3-MC+BX gruplarındaki ortalama alyuvar hacmi (MCV) diğer gruplarla karşılaştırıldığında önemli bir azalma gösterdi (P≤0.05). Sonuç olarak, 3-MC'ye maruziyet durumunda BX'ın uzun sureli oral kullanımı oksidatif stresi azaltamayabilir, WBC, %LY, %NEU, PLT, HGB, HCT, MCV gibi hematolojik parametreleri değiştirebilir. Fakat bu değişimler fizyolojik sınırlar içersinde kalır. Yinede BX'ın kullanımında demir kullanımına dikkat edilmelidir. Ayrıca, bu dozda BX'ın kullanımı inflamasyon ve kanser göstergeleri olan TNF-α, IL-1β, IL-6 seviyelerini azaltabilir.

Anahtar sözcükler: *Boraks, Hematoloji, İnflamasyon, İnterlökin, Sıçan, Tas, Tos, Tnf-α, 3-MC*

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INTRODUCTION

Boron (B) is an essential element being responsible in metabolic reactions, which affect physiological systems of organism. Borax (BX) mineral being a component of Boron on the other hand, is a boric acid salt [1]. Used especially in pharmaceutical industry, Borax is a necessary mineral as a trace element for human, animals and plants [2].

Borax affects activity [3], mineral metabolism (Ca and P) [4], hormones [5] and lipid metabolism [6], free radicals of many enzymes [7,8].

Being one of the unsaturated aromatic hydrocarbons, 3-methylcholanthrene (3-MC) is a chemical carcinogenic which is used in experimental studies. Therefore, it can be applied through hypodermic, peritoneal spread and oral ways to test animals [9].

Tumor necrosis alpha-factor (TNF-α), interleukin-6 (IL-6) and interleukin-1 beta (IL-1β) are released by adipositis. Adipositis can initiate tumor formation in angiogenesis and cancer cells [10]. It is reported that these formations occur by means of cytokines such as TNF-α, IL-6, ve IL-1-receptor agonist [10,11].

Several pro-inflammatory cytokines released by innate and adaptive immune cells have been shown to regulate cancer cell growth and thereby contribute to tumor promotion and progression [12].

There is a remarkable balance between antioxidants and oxidants in a healthy body. Health problems occur due to a rise of free radicals or fall of antioxidants. Total antioxidant status (TAS) and total oxidant status (TOS) measurement is the most practical, economic and rapid practice that detects oxidant [13] and antioxidant [14] amounts.

There is limited information about whether any effects of BX on inflammation, TAS- TOS and haemataological parameters in rats could be applied 3-MC. Therefore, in the present study, we have investigated the effect of BX on TNF-α, IL-1β, IL-6, TAS, TOS, WBC, RBC, PLT values in rats applied 3-MC.

MATERIAL and METHODS

Study Groups

In this study were used total of 24 Wistar albino rats weighing between 200 and 250 g in a climate-controlled animal care facility, with a 12 h light/dark cycle. The animals were given with standard rat chow and water, *ad libitum*.

They were divided into 4 groups each containing 6 rats. Group 1 (Control) was separated as a control group and saline (1 mL of 0.9% NaCl) was injected twice a week first 2 weeks of study with i.p. way. Group 2 (3-MC) was applied 25 mg/kg dosage of 3-MC (Sigma- Aldrich Code: 213942) 2 twice a week first 2 weeks of study with i.p. way and was given normal drinking water during 150 days. Group 3 (BX) was given BX (Aldrich, Code: 2 21732) 300 mg/L/day dose with drinking water, during 150 days. Group 4 (3-MC+BX) was applied 25 mg/kg dosage of 3-MC twice a week first 2 weeks with i.p. way and BX was given 300mg/L/day dose with drinking water, during 150 days. All applications were simultaneously begun first day of study.

This study was approved by the local ethics committee of Yuzuncu Yil University (YUHADEK-Approval No: 2014/5).

Blood Collection

At the end of 150 days, blood samples were collected from all the rats and sacrificed by anesthetizing with a i.p. injection of 70 mg/kg of ketamine HCl (Ketalar, Pfizer) and xylasine HCl 10 mg/kg i. p. xylazine (Rompun, Bayer)

Blood samples were taken from hearts with sterile injector and placed into tubes with EDTA and coagulated tubes. Then bloods were separated into serum by centrifugation at 1.800 g (3.000 RPM) for 10 min. Serum was stored (-20°C) until the analysis.

Assay

The TNF-α, IL-1β, IL-6 levels were analysis by ELISA kits (eBioscience, Austria); TAS, TOS values using a novel automated measurement method developed by Erel [13,14] by colorimetric kits (Rel Assay, Türkiye) in serum. The oxidative stress index (OSI) was calculated with the ratio of TOS to TAS.

Hematology parameters, WBC, % leukocyte, RBC, HGB, HCT, PLT, were determined using rat mode of veterinary the blood cell counter (Abocus Junior Vet-5, Austria) in whole blood.

Statistical Analysis

All data were analyzed using the Kruskal-Wallis test. Dunn test was performed to determine the different groups. Statistics Calculator taken as 5% level of significance and SPSS statistical software 16.0 for Windows was used for the calculations. The data was given as means±stantard deviation (X±SD)

RESULTS

The serum levels of TNF-α, IL-1β and IL-6 are shown in *Table 1*. According to the *Table 1* the levels of TNF-α ($P \leq 0.05$), IL-1β ($P \leq 0.05$) and IL-6 in group 2 increased compared to other groups. But increase of IL-6 was not statistically significant.

The hematolojical parameters are shown in *Table 2*. According to the *Table 2* the increase of WBC levels in group 2 were not significantly compared to other groups. LY% levels of every 3 groups were significantly higher ($P \leq 0.05$);

however, its NEU% (P≤0.01) and MO% were lower than control group. But, MO% was not statistically significant. The HGB and HCT values of group 4, MCV in group 2 and group 4 were obtained significantly decrease (P≤0.05) compared to other groups. The PLT counts in groups 2 and 4 were determined significantly higher than others groups (P≤0.05) *(Table 2)*.

The serum levels of TAS, TOS and OSI are shown in *Table 3*. According to the table 3 serum TOS and OSI levels in groups 2 and group 4 were determined significantly higher (P≤0.01) than other groups. There was no difference for TAS among the groups.

DISCUSSION

Being of great importance for environmental health, 3-MC changes metabolism and toxicity of physiological substances and drugs and leads to mutation after being taken into body. As a result of genotoxic effects of 3-MC, teratogenicity, leucemia, especially lung and cervix cancer types occur.

Cytokines are multi-functional polypeptides which are synthesized by various cells in body and have significant roles in the development of cellular, humoral immune and

Table 1. *Serum TNF-α, IL-1β and IL-6 levels in all the groups (mean±SD)*
Tablo1. *Tüm gruplardaki serum TNF-α, IL-1β and IL-6 seviyeleri*

Inflammation Markers	Control Group n:6	3-MC Group n:6	BX Group n:6	3-MC+BX Group n:6	P Value
TNF-α (pg/mL)	309.76±24.83^{b}	379.70±44.37^{a}	312.92±34.11^{b}	305.31±67.45^{b}	≤0.05
IL-1β (pg/mL)	301.72±95.76^{b}	481.85±79.06^{a}	365.94±80.08^{b}	339.70±89.88^{b}	≤0.05
IL-6 (pg/mL)	162.88±41.12	184.46±47.37	163.22±39.91	157.28±22.73	≥0.05

^{a,b} in the same line values with different letters show statistically significant differences

Table 2. *The haematolojical parameters in all the groups (mean±SD)*
Tablo 2. *Tüm gruplardaki hematolojik parametreler*

Haematolojical Parameters	Control Group n:6	3-MC Group n:6	BX Group n:6	3-MC+ BX Group n:6	P Value
WBC (10^9/L)	6.81±1.93	7.74±1.13	6.53±1.78	6.02±1.88	≥0.05
LY (%)	64.87±9.84^{b}	74.82±4.05^{a}	78.40±2.48^{a}	77.57±2.17^{a}	≤0.05
MO (%)	6.98±3.98	2.96±2.17	3.03±2.05	4.83±4.46	≥0.05
NEU (%)	28.13±6.29^{a}	22.18±2.79^{b}	18.57±2.69^{b}	17.58±3.88^{b}	≤0.01
RBC (10^{12}/L)	7.70±0.19	7.63±0.47	7.52±0.90	7.13±0.76	≥0.05
HGB (g/dL)	13.93±0.37^{a}	13.58±0.53^{a}	13.98±0.66^{a}	12.72±0.79^{b}	≤0.05
HCT (%)	45.74±1.34^{a}	43.80±2.45^{a}	44.43±5.55^{a}	40.71±4.18^{b}	≤0.05
MCV (fl)	59.50±1.52^{a}	57.60±1.49^{b}	59.17±2.23^{a}	57.33±1.37^{b}	≤0.05
MCH (pg)	18.10±0.30	17.88±0.49	18.88±3.47	17.95±1.66	≥0.05
MCHC (g/dL)	30.52±0.82	31.10±0.63	31.98±5.71	31.42±2.83	≥0.05
RDWc (%)	14.83±0.37	14.94±0.71	14.22±0.48	14.97±0.74	≥0.05
PLT (10^9/L)	607.00±69.57^{c}	715.83±71.37^{b}	605.50±75.63^{c}	779.17±67.81^{a}	≤0.05
PCT (%)	0.47±0.22	0.51±0.08	0.44±0.05	0.59±0.14	≥0.05
MPV (fl)	7.85±0.74	7.72±25.14	7.27±0.12	7.53±0.38	≥0.05
PDWc (%)	34.85±1.23	35.05±0.96	34.63±0.44	35.27±0.86	≥0.05

^{a,b,c} in the same line values with different letters show statistically significant differences

Table 3. *Serum TAS-TOS and OSI values in all the groups (mean±SD)*
Tablo 3. *Tüm gruplardaki Serum TAS-TOS and OSI değerleri*

Oxidant-Antioxidant Parameters	Control Group n:6	3-MC Group n:6	BX Group n:6	3-MC+BX Group n:6	P Value
TAS (mmol Trolox Equiv/L)	0.54±0.09	0.52±0.06	0.47±0.7	0.56±0.03	≥0.05
TOS (μmol H_2O_2 Equiv/L)	4.83±1.03^{c}	9.17±1.56^{b}	4.57±1.35^{c}	16.16±2.08^{a}	≤0.01
OSI (Arbitrary Unit)	0.89±0.13^{c}	1.76±0.36^{b}	0.96±0.28^{c}	2.89±0.42^{a}	≤0.01

^{a,b,c} in the same line values with different letters show statistically significant differences

inflamatuar responses; supervising the cell growth and differentiation and initiating cicatrisation processes [1,15]. Main cytokines being responsible for chronic inflammation are TNF-α, IL-6 and inflammasome-activated IL-1β; and TNF-αs and IL-6 play a significant role in cell growth and differentiation [16].

It has been emphasized that IL-6 being a significant cytokine that plays role in inflamatuar response and pathogenesis of cancer [17] is a remarkable marker of experimental cancer, IL-6 levels rise in some cancer patients [18] and anti-apoptotic effects are observed in tumor cells [12].

In a study [9], serum IL-6 and TNF-α levels were investigated in fibrosarcoma induced by 3-MC (0.2 mg). The experiment took about 150-210 days until the appearance of tumor tissue in mause. IL-6 and Tnf-α was higher than controls. In another study, 1 mg of 3-MC was injected into rats with i.p. way; it was determined that it leads to tumor with 66.6% rate and it was reported that 3-MC plays role in cancer biology by means of mutation directly or immune system depression indirectly [19]. In this study, levels of serum TNF-α and IL-1β (P≤0.05), IL-6 in 3 MC group was higher than other groups; these values in BX+3MC group were smiliar to control group.

3-MC injected into rats with 30 mg/kg dosage leads to the synthesis of oncogenic proteins that can be used for the diagnosis [20,21]. In a study [22] detected that 3-MC injected with 200 mg/kg dosage leads to atrophy in thymus glan, T and B lymphositopeny and cancer. However, in these study, ever 3 groups were demonstrated nötropenia (P≤0.01) and lenfositosis (P≤0.05) according to control group. This finding could result from a chronic inflammation which was a result of 3-MC effect. As a matter of fact, it was reported that TNF-α has a toxic effect on β cells of pancreas, ensures vein adhesion of inflamatuar cells, matures monocyte and macrophages and B and T lymphocytes [23,24]. In addition, IL-1 increases expression of surface molecules which help the aggregation of leucocytes; does not directly activate inflammatuar leucocytes as neutrophile does, but affects mononuclear and endothelium cells instead; thus leads to the synthesis of chemokines that activate leucocystes [15,25].

IL-1 has also many inflammatuar characteristics of TNF. For example, it was reported that IL-1 affects endothelium cells and increases coagulation [15,25]. In these study, the PLT counts in 3-MC and 3MC+BX groups were determined to be significantly higher (P≤0.05) while the MCV values significantly lower (P≤0.05) than others groups. At the same time, HGB ve HCT values in 3MC+BX group was significantly decrease according to others groups (P≤0.05). However, these decreases were found to be within physiological limits. Furthermore, decrease of HGB and HCT and increase of PLT may be caused by iron deficiency. Although iron is an essential element for hemoglobin the free iron is moved binds to the transferin, stored as proteins such as ferritin or hemosiderin complexes, it is used holding in the hemoglobin and myoglobin. Because free iron is toxic for cells [26].

Oxidative stress is the imbalance between free radicals and antioxidant defense systems and associated with the etiology and progression of aging and many diseases [27-29]. Having genotoxic effects, 3-MC increases oxidative stress as well [30]. It is asserted that antioxidants taken through nutrition may decrease tumor incidents of antioxidants [20]. Anti-mutagens and antioxidants decrease oxidative stress and lead to decrease in genotoxicity and cancer risk [31].

Studies have demonstrated that B compounds are effective in maintaining the balance of prooxidants and antioxidants by reducing tissue damage resulting from oxidative stress [3,7,32]. Pawa and Ali [7] demonstrated that B limits oxidative damage by enhancing the glutathione store or inducing other free radical elimination.

In our study, TOS values were analyzed to assess the total effect of oxidants. Likewise, we measured the TAS level instead of evaluating antioxidant molecules separately.

In a study [32], B compounds supplementation in diet (100 mg/kg) significantly decreases the lipid peroxidation (LPO) and malondialdehit (MDA) concentration, and enhances the antioxidant defense mechanism such as GSH in blood. However, in this study, serum TOS ve OSI levels in 3MC and 3MC+BX (300 mg/L) groups were determined significant increase according to others groups (P≤0.01). This increase may be from iron deficiency in HGB and increase free iron in blood plasma. In this case free radicals and oxidative stress is increase [33]. Also this stuation may be due to the difference in dose. Turkez et al.[8] reported that B did not alter MDA concentration at low doses (5-50 mg/L) but increased it at high doses (500 mg/L) in human peripheral blood. However, in this study this level of B is nontoxic. Because B compounds are given orally to animals for a short term, the LD50 values for borax in laboratory animals are in the range of approximately 400-700 mg B/kg of body weight [34,35]. Furthermore, the maximum tolerable level of B is 150 mg/kg; diet B deficiency may occur in animals when their diet contains B at 0.3 mg/kg [36].

Antioxidant capacity is an important factor in all physiological standards, and for the performance of humans and all animals [37,38]. Turkez et al.[8] observed that at low doses (15 mg/L) B compounds increased both SOD and CAT activities, while at high doses decresed (500 mg/L) in erythrocytes. Koç et al.[39], were demonstrated that B compounds (100 mg/kg), increases antioxidan capacity in spinal cord ıschemia/reperfusion injury. However, in the present this study, serum TAS levels did not alter in between groups. This result is consistent with literature [32]. Ince et al.[32] showed that dietary B supplementation did not alter the plasma antioxidant capacity when compared to control. Turkez et al.[8] determined TAA in erythrocytes under *in vitro* conditions while we measured it in plasma,

which contains many nonspecific antioxidants such as urea, uric acid, and proteins [32].

According to these studies, the use of BX different doses and time has been reported that its different effects are on oxidative stress and the antioxidant status, but it has not revealed their impact on inflammation markers and the haematolojical parameters. Therefore these effects of BX were evaluated in this study.

In summary in the present study, TNF-α and IL-1β ($P \leq 0.05$), IL-6 ($P \geq 0.05$), WBC ($P \geq 0.05$) levels in 3-MC group, TOS and OSI ($P \leq 0.01$), PLT ($P \leq 0.05$) levels in 3-MC and 3-MC+BX groups were detected increases compared with other groups. It was determined that LY% levels of ever 3 groups were increased ($P \leq 0.05$); however, NEU% ($P \leq 0.01$) and MO% ($P \geq 0.05$) levels were decreased according to control group. Also, MCV in 3-MC and 3-MC+BX groups ($P \leq 0.05$), HGB and HCT values in 3-MC+BX group were decrease a physiology limited compared to other groups ($P \leq 0.05$).

As a result, this experimental study has demonstrated that 3MC may increase the level of inflammation and cancer markers, oxidative stress and some haematolojical parameters. In case of exposure to 3-MC, use alone of BX with 300 mg/L/day dosage with drinking water during 150 days does not decrease oxidative stress, may changes haematolojical parameters such as, WBC, LY%, NEU%, PLT, HGB, HCT, MCV. However, these changes remain within physiological limits. Even so, iron metabolism should be considered in the use of BX. Furtermore, BX with the abovementioned dosage may be used to reduce the levels of TNF-α, IL-1β, IL-6 being inflammation and cancer markers.

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