

Letter to the editor:

GENOTOXICITY EFFECT OF METHYL-TERTIARY BUTYL ETHER ON RAT LYMPHOCYTES USING COMET ASSAY

Shima Alishahi, Zahra Zنده-Boodi, Mostafa Saadat*

Department of Biology, College of Sciences, Shiraz University, Shiraz 71467-13565, Iran

* **Corresponding author:** Mostafa Saadat, Department of Biology, College of Sciences, Shiraz University, Shiraz 71467-13565, Iran, Fax: +98-71-32280926;
E-mail: saadat@shirazu.ac.ir

<http://dx.doi.org/10.17179/excli2020-2159>

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>).

Dear Editor,

Methyl-tertiary butyl ether (MTBE), an oxygenated compound, is added to gasoline in order to reduce air pollutants. MTBE alters enzyme activity or mRNA levels of antioxidant enzymes (Elovaara et al., 2007; Khalili et al., 2015; Badr et al., 2016; Badr and Saadat 2016, 2018) and oncogenes (Zhou et al., 1999). It has been reported that elevation of cancer incidence is associated with exposure to MTBE (Belpoggi et al., 1995). Therefore, its usage has been limited in several countries. However, it is still used in several Middle East countries. In many countries, people are exposed to very low levels of MTBE by drinking contaminated water (Brown 1997). In a majority of previous reports, researchers have investigated the effects of high or very high doses of MTBE on experimental animals (de Peyster et al., 2014; Dongmei et al., 2009; Khalili et al., 2015; Badr et al., 2016). It should be noted that low levels of MTBE significantly alter the gene expression levels of GSTs (Badr and Saadat 2018).

Although many investigations concerning genotoxicity of MTBE and its metabolites have been carried out, the potential risk of human mutagenicity, is still a matter of debate among toxicologists (Bogen et al., 2015). The purpose of the present experiment is to examine the potential of MTBE at low doses in inducing DNA damage condition using alkaline comet assay.

A total of 24 adult Wistar male rats (180–200 g) were purchased from the animal house of Shiraz University of Medical Sciences (Iran). Animals were housed in plastic cages under standard animal house conditions with a 12 h light/dark cycle and a temperature of 25 ± 2 °C, received standard pellet food, and tap water was available *ad libitum*. After acclimation period (10 days), the animals were randomly divided into four equal experimental groups. Group 1 (control) was treated with distilled water and groups 2, 3 and 4 received 5, 10 and 20 mg/kg/day MTBE in distilled water, respectively. The treatments were done through oral gavage for 30 consecutive days.

At the end of the exposure period, animals were anesthetized with ether and blood samples were obtained from their heart. Lymphocytes were separated by density centrifugation at $1000 \times g$ for 30 min at the room temperature over a layer of Ficoll and then the cell suspension was adjusted to 10^4 - 10^6 cells/ml. For positive control, 50 μ L of the cell suspension obtained from untreated rat was mixed with 1 μ M H₂O₂ and incubated for 5 min at 4 °C.

The comet assay was performed under alkaline conditions as described previously (Olive and Banáth, 2006) with some modifications. Thereafter, images of 50 randomly chosen cells were taken per sample with Nikon fluorescence microscope, and then, indices of cellular DNA damage were measured using TriTek CometScore V 2.0 software. Indices of DNA damage were the tail length (TL), tail DNA percent (TD), and tail moment (TM).

Statistical analysis indicated that the TL, TD and TM indices in the positive controls which were treated with 1 μ M H₂O₂ showed maximum levels of DNA damage, while the control group revealed very low DNA damage. One-way analysis of variance demonstrated that there were significant statistical differences between the experimental groups for the LT (F=31.19; df=3, 20; P<0.001), TD (F=22.22; df=3, 20; P<0.001), and TM (F=20.35; df=3, 20; P<0.001) indices. The post hoc Duncan test revealed that the means of the study indices were significantly increased in the groups that received 10 and 20 mg/kg/day MTBE compared with the control levels (Figure 1). Pearson correlation analysis revealed that there were significant positive correlations between MTBE concentrations and the TL (r=+0.882, df=22, P<0.001), TD (r=+0.851, df=22, P<0.001) and TM (r=+0.852, df=22, P<0.001) indices. It means that the amount of DNA damage increases as a function of MTBE concentration.

MTBE has been recently reported to induce chromosomal aberration in bone marrow cells of rats, orally administered with this chemical at high concentration (Darwish and Mosallam 2019). A significant increase in the extent of DNA damage was observed in human lymphocytes treated with MTBE at low doses (Chen et al., 2008). Our present findings indicate that MTBE can induce DNA strand breaks even at low doses under *in vivo* condition.

Considering that MTBE is metabolized to the known weak carcinogens, tert-butanol and formaldehyde (Belpoggi et al., 1995), and in order to find the real association between low levels of MTBE and possible alterations in incidence rates of human cancers, further research in this field is required.

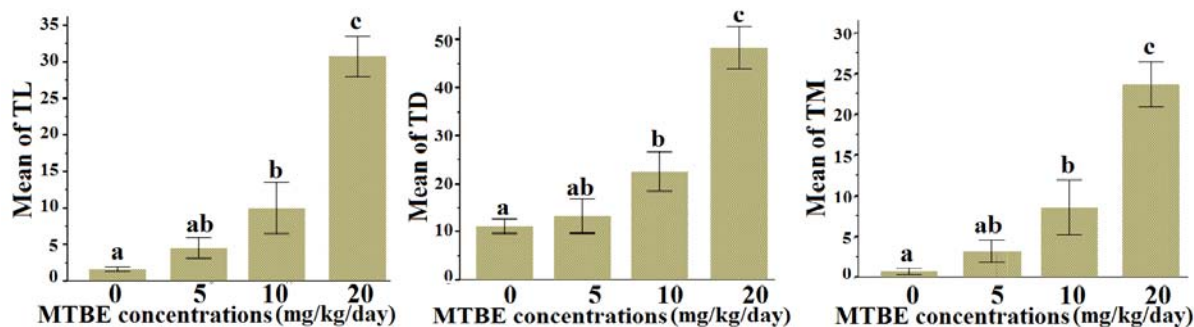


Figure 1: Effects of MTBE on DNA damage indices including the tail length (TL), tail DNA percent (TD), and tail moment (TM). Values are means \pm SE. Same alphabet means no significant differences between groups.

Acknowledgment

This work was supported by the Shiraz University (97GCU2M1741), Iran.

Conflicts of interest

None.

REFERENCES

Badr AA, Saadat M. Expression levels of some detoxification genes in liver and testis of rats exposed to a single dose of methyl-tertiary butyl ether. Maced J Med Sci. 2016;4:232-5.

- Badr AA, Saadat M. Effects of acute and sub-chronic exposure to low doses of methyl-tertiary butyl ether on mRNA levels of three members of glutathione S-transferases in liver and testis of the male rats. *Iran J Public Health*. 2018;47:931-3.
- Badr AA, Saadat I, Saadat M. Study of liver function and expression of some detoxification genes in the male rats exposed to methyl-tertiary butyl ether. *Egypt J Med Hum Genet*. 2016;17:325-9.
- Belpoggi F, Soffritti M, Maltoni C. Methyl-tertiary-butyl ether (MTBE) - a gasoline additive-causes testicular and lymphohaemato-poietic cancers in rats. *Toxicol Ind Health*. 1995;11:119-49.
- Bogen KT, Heilman JM. Reassessment of MTBE cancer potency considering modes of action for MTBE and its metabolites. *Crit Rev Toxicol*. 2015;45(Suppl 1):1-56.
- Brown SL. Atmospheric and potable water exposures to methyl tert-butyl ether (MTBE). *Regul Toxicol Pharmacol*. 1997;25:256-76.
- Chen CS, Hseu YC, Liang SH, Kuo JY, Chen SC. Assessment of genotoxicity of methyl-tert-butyl ether, benzene, toluene, ethylbenzene, and xylene to human lymphocytes using comet assay. *J Hazard Mater*. 2008;153:351-6.
- Darwish IAE, Mosallam SAE. Chromosome aberrations in bone marrow cells of rats treated with MTBE. *Pak J Pharm Sci*. 2019;32:89-93.
- de Peyster A, Mihaich E, Hyung Kim D, Elyea WA, Nemeč MJ, Hirakawa BP, et al. Responses of the steroidogenic pathway from exposure to methyl-tert-butyl ether and tert-butanol. *Toxicology*. 2014;319:23-37.
- Dongmei L, Yi G, Chun-Tao Y, Yu-Feng H, Xiao-Dong H. Effects of sub-chronic methyl tert-butyl ether exposure on male Sprague-Dawley rats. *Toxicol Ind Health*. 2009;25:15-23.
- Elovaara E, Stockmann-Juvala H, Mikkola JV, Gelboin H. Interactive effects of methyl tertiary-butyl ether (MTBE) and tertiary-amyl methyl ether (TAME), ethanol and some drugs: Triglyceridemia, liver toxicity and induction of CYP (2E1, 2B1) and phase II enzymes in female Wistar rats. *Environ Toxicol Pharmacol*. 2007;23:64-72.
- Khalili L, Gholami S, Ansari-lari M. Evaluation of offspring sex ratio, sex hormones and antioxidant enzymes following exposure to methyl tertiary butyl ether in adult male Sprague-Dawley rats. *EXCLI J*. 2015;14:75-82.
- Olive PL, Banáth JP. The comet assay: a method to measure DNA damage in individual cells. *Nat Protoc*. 2006;1(1):23-9.
- Zhou W, Huang G, Zhang H. Effect of methyl tertiary butyl ether on the expression of proto-oncogenes and function genes. *Wei Sheng Yan Jiu*. 1999;30:137-8.