

ORIGINAL ARTICLE

The Impact of Noise and Formaldehyde Exposure on Oxidative Stress Indices in Blood and Liver Tissue of Rat

MOHAMMAD JAVAD JAFARI¹, ALI DEHGHANI^{2*}, ALI KHAVANIN³, MANSOUR AZARI REZA ZADE⁴, ALI DADASHPOURAHANGAR⁵

^{1, 2, 4*} School of Public Health, Department of Occupational Health Engineering Shahid Beheshti University of Medical Sciences, Tehran, Iran; ^{3, 5} Faculty of Medicine, Tarbiat Modaress University, Tehran, Iran;

Received October 4, 2013; Revised March 11, 2014; Accepted March 25, 2014

This paper is available on-line at http://ijoh.tums.ac.ir

ABSTRACT

Simultaneous exposure of highly-used chemical and physical agents are expected to happen in many occupations. In the present study the impact of simultaneous exposure to noise and formaldehyde on oxidative stress in blood and liver tissue of rat was investigated. Animal study was conducted in the School of Public Health, Shahid Beheshti University of Medical Scineces, Tehran, Iran in 2013. A total of 42 male Wistar albino rats were randomly divided into 6 groups (7 in each group). Rats in control group were not exposed to any stressor, while 2 groups were exposed to 6 and 12 ppm formaldehyde, 1 group to noise (0.7-5.7 kHz, 100 dB SPL) and 2 groups to noise + formaldehyde (8 h/d, 28 days). The level of oxidative stress in blood and liver tissue were determined through GSH and MDA measurements. Sound pressure level was monitored using a calibrated Bruel and Kjaer 2238 sound level meter (Denmark). Formaldehyde concentration was monitored four times an hour by a photo ionization detector (Photocheck +5000, lonscience Co., UK). GSH concentrations were measured through titration and quantification of thio-nitro-benzene using spectrophotometry at 412 nm. MDA levels were quantified by absorption at 535 NM wavelengths using a spectrometer. The results revealed that exposure to both stressors significantly reduced the GHS levels and significantly increased the MDA levels in exposed rats. The level of changes in groups with simultaneous exposure was dose-dependent (p < 0.05). The current study clearly confirmed that co-exposure to noise & formaldehyde has an additive effect in oxidant /antioxidant system imbalance.

Keywords: Noise, Formaldehyde, Rat, Malondialdehyde, Glutathione

INTRODUCTION

Formaldehyde (FA) is used mainly as an intermediate product in the manufacture of synthetic resins and their applications, such as adhesives and binders in wood product, furniture, pulp and paper, synthetic vitreous fiber industries and in medical

applications as a disinfectant and Preservative agent [1-3]. High levels of occupational exposure to inhaled FA (6-12 ppm) has been reported that may induce liver toxicity [2, 4]. The International Agency for Research on Cancer (IARC) reclassified FA as a human carcinogen (group 1) in 2009. Adequate evidences show that formaldehyde causes nasopharyngeal cancer and leukemia [4-5]. Moreover, several investigators such as Sogut et al. [6], Zho et al. [7], and Kum et al. [8] have

^{*} Corresponding author: Ali Dehghani,

Email:Dehghani.herfee@gmail.com

	$\mathbf{W}_{\mathbf{body}}, \mathbf{gr}$				$\mathbf{W}_{\mathbf{liver}}$ / $\mathbf{W}_{\mathbf{body}}$		
	Before exposure		After exposure		After exposure		
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	
С	200.6 (17.6)	170-226	227.5 (16.0)	203-246	0.047 (0.002)	0.044-0.049	
Ν	194.9 (13.9)	185-222	220.0 (14.9)	203-241	0.034 (0.002)	0.033-0.038	
FA6	208.6 (19.2)	175-228	200.0 (19.2)	173-220	0.032 (0.001)	0.029-0.033	
FA12	207.6 (17.6)	186-235	185.0 (21.0)	152-207	0.030 (0.002)	0.028-0.034	
FA6+N	196.4 (17.4)	173-218	186.0 (13.0)	165-199	0.030 (0.002)	0.028-0.033	
FA12+N	204.6 (15.2)	182-226	190.7 (11.8)	174-205	0.027 (0.001)	0.026-0.030	

Table 1. The body weight of subjects in studied groups before and after exposures (n=7)

reported that exposure to formaldehyde is associated with oxidative stress in many organisms [9].

It has been confirmed that loud noise not only leads to hearing loss but also may harm other body organisms. In addition to hearing loss, other damaged factors may include oxidative stress, vascular changes, mechanical trauma, etc. [10]. Mbuligwe demonstrated that the noise intensity level of wood-metal industries in a developing country was higher than permissible exposure level recommended by US-OSHA [11]. The analysis of data obtained from human studies and NIHL revealed that long term exposure to noise leads to unusually high levels of ROS (Reactive Oxygen Species) in exposed subjects [12]. Aravind and Manikandan reported that short and prolonged exposure to loud noise produces excessive free radicals and induces disorders in organs such as cardiovascular, nervous and endocrine systems in addition to auditory organs [13-14].

The impact of noise and organic solvents on auditory organs of animals have been studied in a large number of experimental investigations. The synergistic impact has also been described in rats subjected to a combined exposure to ethyl benzene and noise, toluene and noise as well as styrene and noise [15-17]. However, the impact of noise and formaldehyde was not recognized in literature. A synergistic impact in oxidative stress indices of subjects exposed to noise and formaldehyde is expected.

The present study was carried out to explore the combined impacts of noise and formaldehyde on oxidative stress indices in blood & liver tissue of rats.

MATERIALS AND METHODS

Animals and Chemicals

Animal study was conducted in the School of Public Health, Shahid Beheshti University of Medical Scineces, Tehran, Iran in 2013. Forty-two adult male albino Wistar rats (from Pasteur Institute, Tehran, Iran) weighting 200 ± 25 g were randomely divided into 6 groups (7 in each group). The groups were divided according to the type of exposure summarized in Table 1. Animals were housed in a 12/12 h light/dark cycle

(light on between 7:00 a.m. and 7:00 p.m.), maintained at 25 ± 2 °C, with a relative humidity of 45-55% and free access to chow and tap water.

All experiments were executed in conformity with the Guide for the Care and Use of Laboratory Animals (US National Institutes of Health Publication No. 80-23, revised 1996). The proposal was approved by the Research and Ethics Committee of Shahid Beheshti University of Medical Sciences, Tehran, Iran.

The number of rats in each group was determined based on the results of previous studies as well as the results of preliminary experiments [13, 14, 18]. The subjects were exposed to noise and formaldehyde, 8 h/day for 28 days. The chamber was ventilated for 12 air changes per hour through an exhaust fan.

Chemicals used in this study were of analytical grade manufactured by Sigma Chemical Co. (St. Louis, MO, U.S.A.) and Merck (AG, Darmstadt, Germany).

Noise Exposure

Conscious animals were exposed to a continuous noise (0.7–5.7 kHz) at 100 dB SPL (Sound Pressure Level). Broadband noise was generated by a computer system using Cool Edit Software to provide OBN (Octave Band Noise) with center frequencies of 1, 2 and 4 kHz. This signal was amplified by an amplifier and fed to speakers located approximately 30 cm above the subjects. Sound intensities were measured at the level of the rat's head by a daily calibrated Bruel and Kjaer 2238 sound level meter (Denmark). The sound pressure level in the chamber was monitored every hour. The variations of more than 1 dB in noise level were adjusted.

Formaldehyde Exposure

FA gas was generated by thermal depolymerization of para-formaldehyde (Merck AG, Darmstadt, Germany) at 70-90 °C according to a method described by Chang et al. [19] Formaldehyde concentration in the exposure chamber was controlled at a rather desirable range by changing the airflow. FA concentration was measured and monitored four times an hour by a photo ionization detector (Photocheck +5000, Ionscience Co.,

The Impact of Noise and Formaldehyde Exposure on Oxidative Stress

Table 2. The T values of comparing average body weight of each group with onley groups before exposure.						
	С	Ν	FA6	FA12	FA6+N	FA12+N
С	-	0.987	0.950	0.971	0.997	0.998
Ν	0.987	-	0.656	0.723	1.00	0.888
FA6	0.950	0.656	-	1.00	0.759	0.998
FA12	0.971	0.723	1.00	-	0.818	0.999
FA6+N	0.997	1.00	0.759	0.818	-	0.944
FA12+N	0.998	0.888	0.998	0.999	0.944	-

Table 2. The P-values of comparing average body weight of each group with other groups before exposure

Table 3. The *P*-values of comparing average body weight of each group before and after exposure

Group	С	Ν	FA6	FA12	FA6+N	FA12+N
<i>P</i> -value	0.001	0.001	0.014	0.001	0.024	0.001

UK). The preciseness of the measurement was controlled according to the 2016 method recommended by the US National Institute of Occupational Health and Safety (NIOSH) with a sensitivity of less than 0.1% ppm [20].

Surgery and Specimen Preparation

Rats were anesthetized by carbon dioxide inhalation and placed in a stereotactic apparatus before decapitation. After blood was taken from the heart, liver was removed and placed in ice-cold saline. Then liver tissues were washed out from contaminating blood with ice-cold buffered saline. They were weighed and homogenized in Tris-HCl buffer (pH 7.4) for one minute at 16000 RPM with a Homogenizer (Ultra Turrax Type T-25-B; IKA Labortechnic, Staufen, Germany).

GSH and MDA level Determination

GSH concentrations were quantified through titration with 0.1 mmol/l 5, 5'-Dithibios (2-nitrobenzoic acid) in a 0.1 mol/l disodium phosphate buffer solution, pH 8. The thionitrobenzene as reduced product was measured at 412 nm wavelength using a spectrometer [21-22]. Liver and serum GSH levels were represented as μ mol/g and mg/dl respectively.

MDA level was quantified by the method described by Wills & Buege et al. [23-24]. For this purpose, reaction of MDA with thiobarbituric acid was performed by incubating over 60 minutes at 95-100°C. Following the reaction, the absorption of MDA+TBA reactive solution was determined spectrophotometrically at 535 nm. Liver and serum results were represented in terms of nmol/g and nmol/l respectively.

Statistical Analysis

All data were remarked as mean \pm S.D. Student-*t* test and ANOVA test were applied to detect the relationship

between different variables using SPSS version 16.0 (SPSS, Cary, NC, U.S.A.). Statistical level of the test was considered to be P value less than .05.

RESULTS

The average±standard deviation of body and liver/body weights of studied groups are shown in Table 1. The results of statistical comparison of the average body (before and after trial) and liver/body (after trial) weight of each group are shown in Table 1.

The results revealed that there was no significant difference (p>.05) between body weights of different groups before exposure (Table 2). The statistical tests showed that exposure to noise significantly increased the body weight of the exposed rats. Mean time, the exposure to FA as well as FA+noise significantly reduced (p<.05) the body weight of the exposed rats (Table 3).

Oxidative stress indices Liver GSH and MDA

The study showed that exposure to noise alone did not significantly reduce (p>.05) the GSH level in liver tissue of rats (Fig 1). Exposure to FA significantly reduced (p<.05) the GSH level in liver tissue of rats (Fig. 1). The influences of FA+noise exposure seems to be synergistic (Fig 1).

The results showed that exposure to noise alone significantly (p<.05) increased the MDA level in liver tissue of rats (Fig 2). The exposure to FA also significantly increased the MDA level in liver tissue of rats. The simultaneous exposure of FA+noise seems to be non synergic (Fig 2). Fig. 2 showes that induced elevation of MDA in liver tissue seems to be dose dependent. Furthermore, stress factors including (noise, FA (6PPM), FA (12PPM), FA(6PPM)+noise & FA(12PPM)+noise) elevated liver MDA levels by 3.09, 5.45, 5.85, 4.67 & 5.54 times respectively.



Fig 1. GHS levels in liver tissue in groups under study (N=7)



Fig 2. MDA levels in liver tissue in groups under study (N=7)

Serum GSH and MDA

According to the results, the serum GSH level in exposed groups significantly decreased compared to the control group (p<.001). GSH level depletion due to FA exposure and noise seems to be synergic (Fig 3). In addition, GSH depletion caused by FA exposure was in a dose-dependent pattern (p<.05, Fig 3).

The results also revealed that serum MDA level significantly increased in exposed groups compared to the control group (p<.001). The statistical analysis showed that the levels of changes in FA exposed groups as well as FA+N groups were not dose-dependent (p>.05). Furthermore, the serum MDA levels of exposed groups (e.g. N, F6, F12, F6+N, F12+N) were elevated by 2.4, 2.59, 2.84, 3.71 & 4.28 times respectively compared to the control group (Fig 4).

DISCUSSION

According to our results, simultaneous exposure to noise and formaldehyde could result in oxidative stress in blood and liver tissue of experimental animals. Exposure to noise in the workplace may accompany chemical solvents such as Formaldehyde. Although, workplace-related noise exposure is considered as a major reason of stress, there are also evidences showing interaction between certain chemicals and noise on oxidative stress [15-17]. However, to our knowledge, no released paper attempted to investigate the interaction of noise and FA on oxidative stress. ROS are generated by cellular metabolic activities and various environmental factors, such as air pollutants, noise [10, 25].

The present study showed that exposure to 6 ppm Formaldehyde for 8 h/day and 28 days significantly decreased the body weight by 3.8% while the exposure



Fig 3. GHS levels in serum of groups under study (N=7)



Fig 4. MDA levels in serum of groups under study (N=7)

to 12 ppm of FA for the same period led to 10.6% body weight loss. In addition, liver to body weight ratio in experimental groups was significantly lower than control group (p<.001). A dramatic loss in total body weight and in selected organ weights of experimental animals is usually considered to be a representative of general toxicity following stress exposure [26]. Body and liver weight decreased after high dose/short term [8] or low dose/long term exposure to FA [27]. Body and liver weight were depressed after 20ppm FA inhalation exposure [28]. The results of the present study well agree with those in literature.

While Monsefi found that noise exposure had no effect on the body and liver weights [29] conversely in Manikandan study, body weight loss was observed after 20-day noise exposure [13]. In the present study the rats exposed to a broad band noise (0.7-5.7 khz) at 100 dB

SPL, gained their weight by 12.9%. Since the control group also gained their weight by 13.4%, it seems that noise had no effects on the exposed subjects.

Almost all previous studies regarding the effect of organic solvents and physical stressores on the activities of antioxidant and xenobotic enzymes have focused on liver or testis, kidney or brain [30-32]. Study on NIHL using animal models has resulted in two primary theories for the fundamental cause. One is that metabolic stress initiates hair cell death [33-34]. Recently metabolic stress theories have focused on the formation of ROS (free radicals) evoked by excessive noise stimulation, pursued by activation of apoptosis signaling pathways to cell death. Immediate appearing of ROS after noise exposure [35] and its persisting for 7-10 days [31], leads to delayed spread of injury, that is a principal characteristic of oxidative stress, as it might

provide a "window of opportunity" for post-exposure intervention and restraint of the extent of injury.

In the present study, after the noise and FA exposure, liver and serum MDA levels in exposed groups had altered dramatically (p<.001). Although MDA elevation resulted in GSH level depletion in serum and liver tissue, but in the noise exposure group, liver GSH remained unchanged (p>.05). Besides the level of GSH and MDA changes in simultaneous groups were dose-dependent (p<.05). In this study, MDA levels were similar to those reported in other studies, which showed that toluene, xylene, Formaldehyde and noise caused an increase in liver and serum MDA significantly [36-40].

Comparison of biochemical parameters of rats exposed to noise, FA or both showed that FA is the major factor for increased oxidative stress. This relationship was also investigated in groups of rats exposed to FA and noise under different experimental conditions [41-42]. Serum GSH decrease in animals exposed to noise may demonstrate that the noiseinduced damage is systematic oxidative stress rather than localized one.

In the present study, depletion of liver GSH levels, as expressed by increase of tissue MDA levels, in rats exposed to FA and a mixture of the two stress sources were observed. This study is the first to evaluate the effects of noise and FA on oxidative stress simultaneously.

CONCLUSION

Four weeks exposure to noise, FA or a mixture of the two is toxic to blood and liver tissue, especially in co-exposure groups. For further research exploring the toxicities of noise and FA simultaneously in occupationally exposed groups is recommended.

ACKNOWLEDGEMENTS

This work was supported by Shahid Beheshti University of Medical Sciences Research Funds with agreement No. p/25/11/2517. The authors declare that there is no conflict of interests.

Nomenclatures

dB, decibel; FA, Formaldehyde; OBN, Octave Band Noise; ppm, Parts per million; GSH, Glutathione; ROS, Reactive Oxygen Species; IARC, International Agency for Research on Cancer; RPM, Rotation Per Minuite; MDA, Malondealdehyde; TBARS, Thio-Barbituric Acid Reactive Substances; NIHL, Noise-Induced Hearing Loss; W_{body}, Body weight; Nm, nanometers; W_{liver}, Liver weight.

REFERENCES

 Pala M, Ugolini D, Ceppi M, Rizzo F, Maiorana L, Bolognesi C, et al. Occupational exposure to formaldehyde and biological monitoring of Research Institute workers. *Cancer Detect Prev* 2008; 32:121-126.

Published online: April 12, 2014

- Franks SJ. A mathematical model for the absorption and metabolism of formaldehyde vapour by humans. *Toxicol Appl Pharmacol* 2005; 206: 309-320.
- 3. Golden R. Identifying an indoor air exposure limit for formaldehyde considering both irritation and cancer hazards. *Crit Rev Toxicol* 2011 September; 41(8): 672–721.
- Zhang L, Steinmaus C, Eastmond D, Xin X, Smith M. Formaldehyde exposure and leukemia: a new meta-analysis and potencial mechanisms. *Mut Res* 2009; 681: 150-168.
- Baan R, Grosse Y, Straif K, Secretan B, Ghissassi FE, Bouvard V, et al. On behalf of the WHO International Agency for Research on Cancer Monograph Working Group, A review of human carcinogens. Part F. Chemical agents and related occupations. *The Lancet Oncol* 2009; 10: 1143-44.
- Sogut S, Songur A, Ozen O. Does the subacute (4-week) exposure to formaldehyde inhalation, lead to oxidant/antioxidant imbalance in rat liver? *Eur J Gen Med* 2004; 1(3): p. 26-32.
- Zhou D, Qiu S, Zhang J. The protective effect of vitamin E against oxidative damage caused by formaldehyde in the testes of adult rats. *Asian J Androl* 2006; 8(5): 584-585.
- Kum S, Sandiksi M, Eren U, Metin N. Effect of formaldehyde and xylene inhalation on fatty Liver and Kidney in adult and developing rats. J Anim Vet Adv 2010; 9(2): 396-401.
- Bolt HM, Morfeld P. New results on formaldehyde: the 2nd International Formaldehyde Science Conference (Madrid, 19–20 April 2012). Arch Toxicol. 2013 January; 87(1): 217–222.
- Haase GM, Prasad KN, Cole WC, Baggett-strehalu JM, Wyatt SE. Antioxidant nicronutrient impact on hearing disorders: concept, rationale and evidence. *Am J Otolaryngol* 2011; 32:55-61.
- 11. Mbuligwe SE. Levels and influencing factors of noise pollution from small-scale industries (SSIs) in a developing country. *Environ Manage* 2004 Jun; 33(6): 830-839.
- Kaygusuz I, Ozturk A, Ustundag B, Yalcin S. Role of free oxygen radicals in noise-related hearing impairment. *Hear Res* 2001; 162: 43-47.
- Aravind KN, Mathangi DC, Namasivayam A. Noise induced changes in free radical scavenging enzymes in the blood and brain of albino rats. *Med Sci Res*1998; 26: 811-0812.
- Pinar T, Atli AK, Alacam H, Omar MB, Amin NA, Akyol O. The Effects of Noise on Oxidative and Antioxidative Balance in Human Erythrocytes. *Int J Hemato Oncol* 2011; 21(1): 27-37.
- Cappaert NL, Klis SF, Muijser H, Kulig BM, Smoorenburg GF. Simultaneous exposure to ethyl benzene and noise: synergistic effects on the outer hair cells. *Hear Res* 2001; 162: 67-79.
- Brandt-Lassen R, Lund SP, Jepsen GB. Rats Exposed to Toluene and Noise May Develop Loss of Auditory Sensitivity due to Synergistic Interaction. *Noise Health* 2000; 3: 33-44.
- Makitie AA, Pirvola U, Pyykko I, Sakakibara H, Riihimaki V, Ylikoski J. The ototoxic interaction of styrene and noise. *Hear Res* 2003; 179: 9-20.
- Angrini, Manar A. Leslie, Julian C. Vitamin C attenuates the physiological and behavioural changes induced by long-term exposure to noise. *Behavio Pharm* 2012; 23(2):119–125.
- Chang JC, Steinhagen WH, Barrow CS. Effect of single or repeated formaldehyde exposure on minute volume of B6C3F1 mice and F-344 rats. *Toxicol Appl Pharmacol* 1981; 61(3):451-59.
- Colli M, Gironi A, Molina V, Marchetti R, Melzi D'Eril G, Lucarelli C. Improved HPLC Methodology in Occupational Exposure Studies on Formaldehyde. *Chromatogr* August 1991; 32(3/4):113-15.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963; 61: 882-88.
- Sedlak J, Lindsay RH. Estimation of total protein bound and non-protein sulphydryl groups in tissue with Ellman's reagent. *Anal Biochem* 1968; 25(1): 192-205.

The Impact of Noise and Formaldehyde Exposure on Oxidative Stress

- 23. Wills ED. Lipid peroxide formation in microsomes: General consideration. *Biochem J* 1969; 113(2): 315-24.
- 24. Buege JA, Aust SD. Microsomal lipid peroxidation. *Methods Enzymol* 1978; 52: 302-11.
- Cho AK, Sioutas C, Miguel AH, Kumagai Y, Schmitz DA, Singh M, et al. Redox activity of airborne particulate matter at different sites in the Los Angeles Basin. *Environ Res* 2005; 99:40–47.
- Duong A, Steinmaus C, McHale CM, Vaughan CP, Zhang L. Reproductive and Developmental Toxicity of Formaldehyde: A Systematic Review. *Mutat Res* 2011 Novemb; 728(3): 118-138.
- Wang F, Li C, Liu W, Jin Y. Oxidative damage and genotoxic effect in mice caused by sub-chronic exposure to low-dose volatile organic compounds. *Inhal Toxicol* 2013; 25(5):235-42.
- Rusch GM, Clary JJ, Rinehart WE, Bolt HF. A 26-week inhalation toxicity study with formaldehyde in the monkey, rat & hamster. *Toxicol Appl Pharmacol* 1983; 68: 329-43.
- Monsefi M, Bahoddini A, Nazemi S, Dehghani GA. Effects of Noise Exposure on the Volume of Adrenal Gland and Serum Levels of Cortisol in Rat. *Iran J Med Sci* 2006; 31(1):5-8.
- Hamada N, Matsumoto H, Hara T, Kobayashi Y. Intercellular and intracellular signaling pathways mediating ionizing radiation-induced bystander effects. *J Radiat Res* 2007; 48:87-95.
- Yamashita D, Jiang HY, Schacht J. Delayed production of free radicals following noise exposure. *Brain Res* 2004; 1019:201-209.
- Sliwinska-Kowalska M, Zamyslowska-Szmytke E, Szymczak W, Kotylo P, Fiszer M, et al. Effects of coexposure to noise and mixture of organic solvents on hearing in dockyard workers. J Occup Environ Med 2004; 46:30-38.
- Hawkins JE, Schacht J. Sketches of otohistory. Part 10: noiseinduced hearing loss. *Audiol Neurootol* 2005; 10:305-309.

- Henderson D, Bielefeld EC, Harris KC, Hu BH. The role of oxidative stress in noise-induced hearing loss. *Ear Hear* 2006; 27:1-19.
- Vlajkovic SM, Lin SC, Wong AC, Wackrow B, Thorne PR. Noise-induced changes in expression levels of NADPH oxidases in the cochlea. *Hear Res* 2013 Oct; 304:145-52.
- 36. Rana SVS, Kumar S. lipid peroxidation in liver, kidney and brain of rats after combined exposure xylene, toluene and methylalcohol. *Indian J Exp Biol* 1994; 32: 919-21.
- Ohlemiller KK, Wright JS, Dugan LL. Early elevation of cochlear reactive oxygen species following noise exposure. *Audiol Neurootol* 1999; 4:229-236.
- Rewerska A, Pawelczyk M, Rajkowska E, Politanski P, Sliwinska-Kowalska M. Evaluating d-methionine dose to attenuate oxidative stress-mediated hearing loss following overexposure to noise. *Eur Arch Otorhinolaryngol* 2013; 270(4): 1513–1520.
- Demirel R, Mollaoğlu H, Yeşilyurt H, Üçok K, Ayçiçek A, Akkaya M. Noise Induces Oxidative Stress in Rat. *Eur J Gen Med* 2009;6(1): 20-24
- Saito Y, Nishio K, Yoshida Y, Niki E. Cytotoxic effect of formaldehyde with free radicals via increment of cellular reactive oxygen species. *Toxicol* 2005. 210: 235-245.
- Njoya HK, Ofusori DA, Nwangwu SC, Amegor OF, Akinyeye AJ, Abayobi TA. Histopathological effect of exposure of Formaldehyde vapour on the trachea and lung of adult wistar rats. *Int J Integ Biol* 2009; 7(3):160-165.
- Lo WC, Liao LJ, Wang CT, Young YH, Chang YL, Cheng PW. Dose-Dependent Effects of D-methionine for Rescuing Noiseinduced Permanent Threshold Shift in Guinea Pigs. *Neuroscience* 2013.09.027. [Epub ahead of print].