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Assessment of carcinogenic benzo[a]pyrene acute toxicity involving oxidative stress in mice lung and liver.

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ABSTRACT/RESUME

Abstract: *Benzo[a]pyrene is one of the most prevalent environmental carcinogen and genotoxic agent. However, the mechanisms of benzo[a]pyrene-induced oxidative damage and acute toxicity in lung and liver tissues especially in mice are still poorly studied. This study was carried out to investigate the effect of short-term exposure to benzo[a]pyrene in mice. Thirty six mice were divided into two main groups: a control group, receiving sunflower oil as benzo[a]pyrene vehicle and an intoxicated group, receiving a unique intraperitoneal injection of 50 mg/kg of benzo[a]pyrene; each group was then divided into three sub-groups and sacrificed at 24h, 48h and 72h after benzo[a]pyrene/vehicle injection. The acute toxicity was assessed by the measurement of oxidative stress parameters: Glutathione content, antioxidant enzymes activity and lipid peroxidation level in both mice lung and liver. The acute exposure to benzo[a]pyrene induces variations in the activity of the enzymatic antioxidants: Catalase and glutathione-s-transferase, a decrease in glutathione content after 24 hours in the mice lung and an increase in lipid peroxidation especially in the mice liver. These results suggest that oxidative stress is actively involved in benzo[a]pyrene acute intoxication in mice.*

I. Introduction

Air pollution is a major problem of recent decades, which has a serious toxicological impact on human health and the environment. The sources of pollution vary from cigarette smoke and natural sources such as volcanic activities to large volumes of emissions from automobiles and industrial activities [1]. In fact, hazardous chemicals escape into the environment by a number of natural and/or anthropogenic activities and may cause adverse effects on human health. Pollution has both acute and chronic effects on the human body, affecting a number of different systems and organs. First, it ranges from minor upper respiratory irritation to chronic respiratory disease, lung cancer and acute respiratory infections; then, pollutants have the capacity to reach the cardiovascular system causing angina, myocardial infarction and increased mortality caused by ischemic heart disease; in the nervous system, they cause neurotoxicity leading to several neuropathies; finally, in the urinary and the digestive system they induce kidney damage such as initial tubular dysfunction and renal cancer, besides liver cell damage, gastrointestinal and liver cancer [2]. Common cellular mechanism by which most pollutants exert their adverse effects is their ability to act directly as prooxidants of lipids and proteins or as free radical generators, promoting oxidative stress and the induction of inflammatory responses [3, 4]. Free radicals (reactive oxygen and nitrogen species) are harmful to cellular lipids, proteins and nuclear or mitochondrial DNA, inhibiting their normal function [5].

Some of the major air pollutants are the polycyclic aromatic hydrocarbons (PAH). They are chemical pollutants that are almost totally produced by industrial processes and human activity and classified as carcinogen and mutagen compounds [1]. Benzo[a]pyrene (BaP) is a prototypical and a well-characterized member of the PAH family [6] and a procarcinogen formed in the process of incomplete combustion of organic materials [7]. Indeed, once taken up into cells, BaP undergoes metabolic activation by the cytochrome P450-dependent monooxygenase system and is converted to reactive, toxic metabolites that bind covalently to cellular elements such as DNA and also generate reactive oxygen species (ROS) which damage cellular macromolecules [8, 9]. Furthermore, it has been reported that BaP derivatives have the capacity to enter redox cycles and induce the production of ROS, causing thereby oxidative stress [10]. However, most of the studies describing the toxic action of BaP *in vivo* are limited to some marine species and only few data of its action in mice are available. Moreover, chronic and long term effects of BaP including carcinogenicity (especially lung carcinogenesis), teratogenicity, neurotoxicity and immunotoxicity has been extensively studied but scarce attention has been accorded to the short term and acute toxicity of this pollutant.

II. Materials and methods

II.1. Chemicals

BaP (purity >96% HPLC), trichloroacetic acid (TCA), thiobarbituric acid (TBA) (≥98%), hydrogen peroxide solution (H_2O_2) (30-31%) and n-butanol were purchased from Sigma Aldrich Co (St. Louis, USA). 1-chloro-2, 6-dinitrobenzene (CDNB) was purchased from Fluka Analytical (Munich, Germany). Glutathion (GSH) and 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from Alfa Aesar (Haverhill, USA). Potassium chloride (KCl) was purchased from Emsure® (Darmstadt, Germany) and phosphate buffers was purchased from Carlo Erba Reagants S.A.S (Val de Reuil, France).

II.2. Animals and husbandry

Male mice weighing 20-25 g were purchased from Pasteur Institute (Algiers, Algeria) and kept in a cross-ventilated room at 22±2°C under relative humidity of 50-60% and light dark cycle of 12 hours. They had free access to standard pelleted food and water. The animals received a minimum of 2 weeks acclimatization period before the beginning of experimental procedures. Experiments on animals were performed in accordance with the guidelines of the Institutional Animal Ethics Committee.

II.3. Experimental protocol

The intoxication was realized on mice weighing 20-25 g by intra-peritoneal (i.p.) injection of 50 mg/kg of BaP (>96% HPLC) dissolved in sunflower oil, the dose was fixed based on the investigation of an acute oxidative stress induced by BaP in mice [11]. The animals being divided into two main groups: The control group (n=18), receiving an equal volume (1 mL/kg) of sunflower oil by i.p. injection and the intoxicated group (n=18), receiving an i.p. unique injection of 50 mg/kg of BaP. Each group was divided into 3 sub-groups of six mice each according to the time required for sacrifice, respectively: 24 hours, 48 hours and 72 hours after the BaP/vehicle injection.

The mice were sacrificed at their respective post i.p. injection of BaP/vehicle required times of 24, 48 and 72 hours by cervical dislocation. The liver and lung were quickly removed, washed in an ice-cold saline solution and stored at - 40 °C in order to evaluate the cellular oxidative stress parameters.

II.4. Measurement of acute oxidative stress in mice lung and liver

The frozen liver and lung tissues samples were quickly weighed (0.2 g) before being homogenized in ice-cold 3 volumes of 100 mM potassium phosphate buffer (pH=7.4) containing 1.15 M KCl. The homogenates were centrifuged at 4000×g for 15 min, at 4°C then the supernatant was centrifuged at 10,000×g for 30 min at 4°C [12]. The supernatants were separated and used for enzymes activity assays. Total protein concentration was estimated by the technique of Bradford [13].

II.4.1. Total Glutathione Estimation

The glutathione (GSH) content in lung and liver was estimated by the method of Ellman [14]. Each reaction consists of 10 mM DTNB [5,5′-dithiobis (2-nitrobenzoic acid)] in 0.1 M sodium phosphate pH= 8.0, supernatant fraction and 0.1 M phosphate buffer. The absorbance was measured at 412 nm and the activity was calculated based on a calibration curve plot using GSH standard.

II.4.2. Antioxidant enzymes activities measurement

The Catalase (CAT) activity was measured as described by Claiborne [15], the assay mixture consists of 0.1 M phosphate buffer (pH 7.2), 19 mM hydrogen peroxide (H_2O_2) and cytosolic fraction. The reaction was carried out at 25 °C and the change in absorbance was recorded at 240 nm. The CAT activity was calculated in terms of µmol H_2O_2 consumed/min/mg protein. The glutathione-s-transferase (GST) activity was determined as described by Habig *et al* [16]. The reaction mixture (0.1 M PBS (pH 6.5) and 20 mM 1-chloro-2, 6-

dinitrobenzene (CDNB)) was preincubated at 37°C and then supplemented with 20 mM GSH and cytosolic fraction. The optical density was measured every minute at 340 nm.

II.4.3. Measurement of lipid peroxidation level

Lipid peroxidation was estimated by using a modified method of Ohkawa [17], 0.5 mL of the homogenate was added to 0.5 ml of trichloroacetic acid (TCA) 20% and 1 mL of thiobarbituric acid (TBA) 0.67%. The mixture was incubated in a boiling water bath for 15 min and, having cooled down, received 4 mL of n-butanol. This mixture was centrifuged at 3000 rpm for another 15 min. The supernatant was collected; absorbance was recorded at 532 nm against the control blank.

II.5. Statistical analysis

Data are presented as means ± SD (standard deviation). Assays were performed in duplicate, and the average values were considered as one independent determination. Statistical differences were determined by unpaired two-tailed student's tests and $p< 0.05$ was accepted as significant difference.

III. Results and discussion

III.1. Effect of BaP intoxication on the GSH content

The amount of GSH reveals variations both among the two groups of mice and according to the organ studied, lung or liver. In the lung, the amount of GSH in the group that is intoxicated 24 hours after the i.p. injection of 50 mg/kg of BaP shows a significant reduction ($p \leq 0.05$) compared with the control group, While the amount of GSH in the liver reveals almost identical results to those previously observed for the lungs, however, the group of intoxicated mice having received 50 mg/kg of BaP does not reveal any significant difference in the GSH content compared to the control group.

48 hours after the intoxication with 50 mg/kg of BaP, the rate of GSH rises in a highly significant manner in the liver ($p \leq 0.01$) as well as in the lungs ($p \leq 0.01$) and it keeps increasing slightly in the hepatic organ 72 hours later ($p \leq 0.001$), whereas, in the lungs, it gets back to a normal value (that of the control group), 72 hours after the i.p. injection of BaP (Figure 1).

III.2. Effect of BaP intoxication on the antioxidant enzymes activity

The CAT enzymatic activity brings out an obvious variation between the two studied organs, mice lung and liver. Indeed, in the lung, there is no significant difference among the two groups of mice: an increase in the activity of the CAT is noted in the group of mice intoxicated with 50 mg/kg of BaP compared with the control mice, but which remains non significant. On the contrary, in the liver, the difference in the enzymatic activity between the two groups of mice is very well marked: the group of BaP-intoxicated mice shows a highly significant increase in the enzymatic activity of CAT compared with the control group ($p \leq 0.001$).

In both the studied organs, the CAT enzymatic activity increases up significantly in the mice lung ($p \leq 0.01$) as well as in the liver ($p \leq 0.001$), 48 hours after the intoxication by an i.p. injection of 50 mg/kg of BaP. Then, after 72 hours from the intoxication induction, the enzymatic activity of CAT gets back to a normal activity similar to that of the control group, in both mice lung and liver.

Our study showed a significant decrease in the enzymatic activity of the GST below its basic level in the liver after the i.p. injection of 50 mg/kg of BaP, conversely, the lung showed a significant increase in the enzymatic activity of GST which displays a decrease in the enzymatic antioxidant capacities and the extension of the BaP effect beyond the 24 hours post injection. In fact, 48 and 72 hours after exposition to 50 mg/kg of BaP, the enzymatic activity of the GST demonstrated a highly significant decrease in the lung ($p \leq 0.001$) as well as in the liver ($p \leq 0.01$) (Figure 2).

III.3. Effect of BaP intoxication on lipid peroxidation

The differences in the amount of cytosolic molondialdehyde (MDA) between the two groups of mice and between the studied groups are obvious, so that the liver seems to show a high level of lipid peroxidation, indeed, the amount of cytosolic MDA in the BaP intoxicated mice is significantly higher (threefold increase) than that of the healthy mice of the control group. In the lungs, the BaP acute intoxication brings about an increase in the cytosolic MDA level but remains non-significant. 48 and 72 hours after the i.p. injection of 50 mg/kg of BaP, the amount of MDA remains high compared with that in the control group. Moreover, this increase is visibly higher in the hepatic organ than in the lung, 48 hours after the injection and keeps rising in this key organ until 72 hours later (Figure 1).

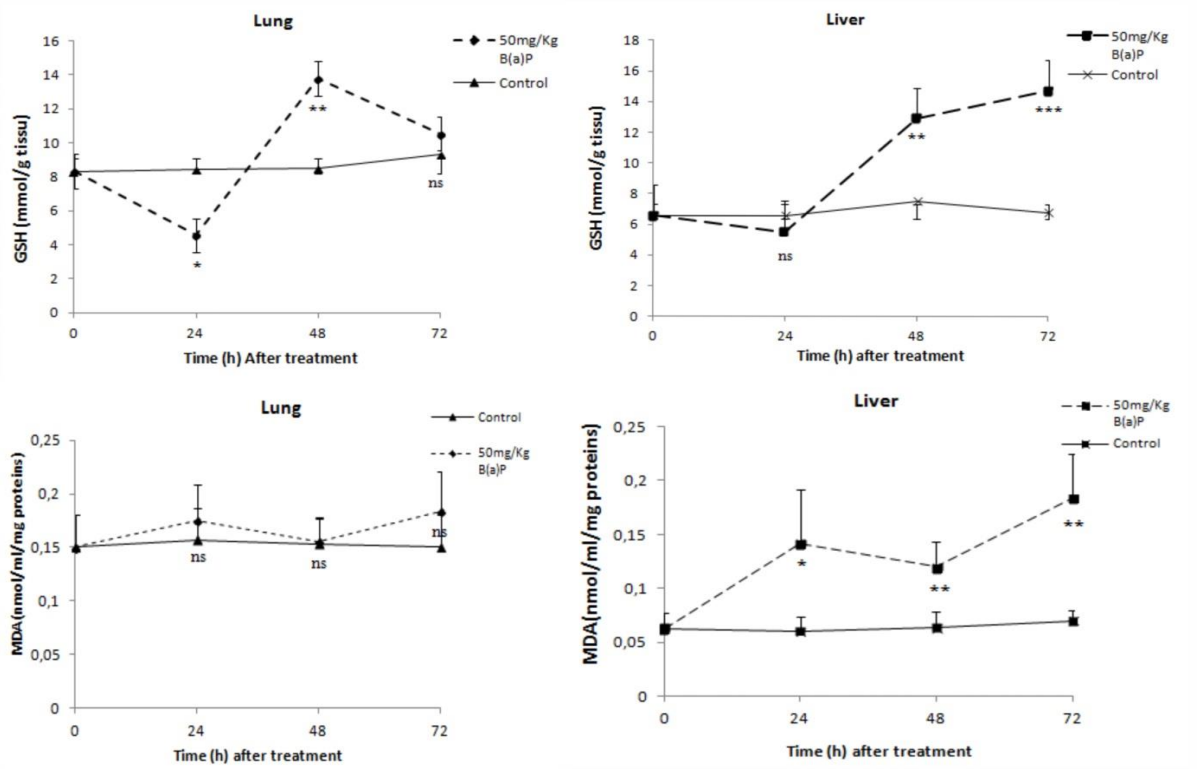

Figure 1. *Acute effects of BaP on the GSH content and lipid peroxidation in lung and liver tissues of mice. Determinations were performed at various post injection time points (24h, 48h and 72 h) after i.p. injection of 50 mg/kg of BaP (the intoxicated group) or sunflower oil (the control group) in mice. Data are expressed as mean ± SD. *** significantly different from control group at P≤0.001; ** significantly different from control group at P≤0.01; * significantly different from the control group at P≤0.05; ns: not significant, from the control group.*

Molecular responses to PAH exposure, mechanisms of PAH uptake and distribution, DNA damage and enzymatic detoxification are under active investigation and BaP is a potent inducer of carcinogenesis mainly through the generation of its metabolites which may directly engender ROS through redox cycling or may disrupt mitochondrial electron transport, leading to more ROS formation. Simultaneously, if the antioxidative defense systems fail to neutralize chemical reactive intermediates produced by endogenous pathways and/or xenobiotic metabolism, we then talk about oxidative stress. For this reason, we investigated here the activities of the antioxidant enzymes, the level of lipid peroxidation and GSH in both two target organs of BaP namely lung and liver, in order to estimate the oxidative stress caused during acute intoxication by this PAH.

In accordance with the study of Hegazy *et al* [18], the BaP acute intoxication induced a reduction of the GSH amount in the mice lung, which is certainly due to an excessive use of this antioxidant for the neutralization of a high concentration of ROS generated in these conditions of toxicity [19]. A reduction in the GSH level was also observed in the liver and it may be due to the fact the liver is the main organ responsible for the process of xenobiotic detoxification, mainly through the presence of a high amount of hepatic GSH (higher than that of the pulmonary level). The increase of the GSH rate in the liver as well as in the lungs, 48 and 72 hours after the BaP intoxication can be explained by the fact that a maximum elimination of BaP and its metabolites by the hepatobiliary system takes place 24 to 48 hours after exposition [20].

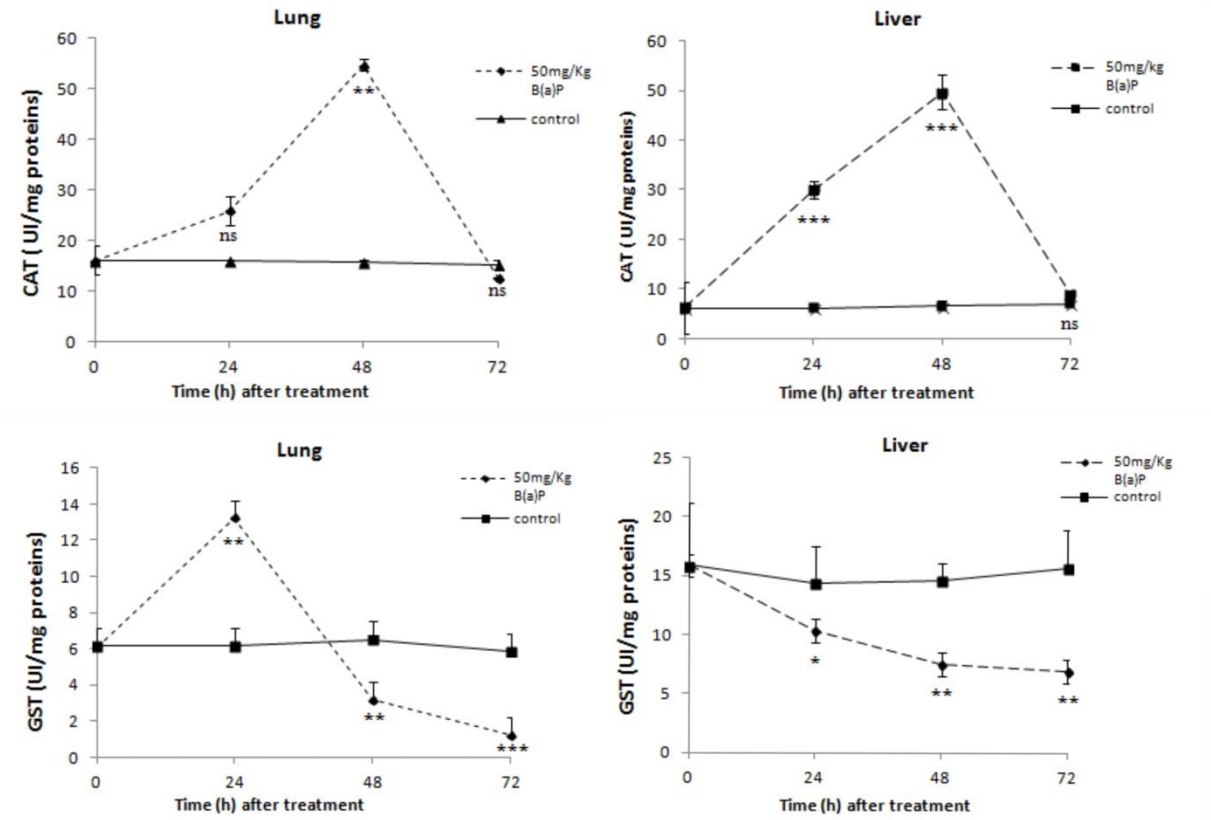

Figure 2. *Acute effects of BaP on the enzymatic activities of CAT and GST in lung and liver tissues of mice. Determinations were performed at various post injection time points (24h, 48h and 72 h) after i.p. injection of 50 mg/kg of BaP (the intoxicated group) or sunflower oil (the control group) in mice. Data are expressed as mean ± SD. *** significantly different from control group at P≤0.001; ** significantly different from control group at P≤0.01; * significantly different from the control group at P≤0.05; ns: not significant, from the control group.*

During its metabolism, BaP is responsible for the production of the ROS such as superoxide anion, the hydroxyl radical and hydrogen peroxide [21] by way of the enzymes of P450 cytochrome. These enzymes being present mainly in the liver implies a more important production of free radicals in the liver than that observed in the lungs, this could explain why the increase of the CAT enzymatic activity is in correlation with the high concentrations of the produced ROS, particularly that of H_2O_2. The enzymatic activity of CAT in the lung is less important than that in the liver because the production of H_2O_2 induced by the acute BaP intoxication is less important. The increase in CAT enzymatic activity 48 hours after the intoxication by BaP is in accordance with other studies, in which the increase of the CAT enzymatic activity is correlated with an increase in the production of H_2O_2 induced by an i.p. injection of 50 mg/kg of BaP. This activity then diminishes to reach a normal value after 72 hours, in both liver and lung, this can be explained by the simultaneous decrease in the production of H_2O_2 by the BaP, CAT being the enzyme responsible for the degradation of H_2O_2.

A preceding study showing that the inhibition of the GST enzymatic activity below its basic level could encourage the production of ROS as well as their effects on the functional, structural integrity of cellular membranes and organites; which will later lead to a reduction of the detoxification capacity of BaP and to an increase in the vulnerability to oxidative stress [11]. In the course of detoxification, the GST goes into competition with the Epoxide hydrolase (mEH) enzyme, both ensuring the detoxification of Benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (the most reactive metabolite of BaP), and any increase in mEH as well as any reduction of the GST activity leads to an increase in the synthesis of this xenobiotic. The increase of enzymatic activity of the GST in lung is certainly due to the occurrence of oxidative stress; the latter inducing the transcription of several genes coding for GST, in order to increase the protection of the cells against GST's harmful effects of this

environmental carcinogen, in this organ. The GST plays a key role in the detoxification of xenobiotics, consequently, a decrease of its enzymatic activity below the normal threshold causes an acceleration in the production of ROS leading to a series of effects on the structural and functional integrity of the cellular membranes as well as the organites [11, 18].

Finally, the increase of MDA level in mice liver can be related to several studies conducted in mice whereby BaP causes an increase of the lipid peroxidation in the liver and kidney tissues in Swiss albinos mice and suggests a sensitivity of these organs to BaP-induced oxidative stress [22]. The increase of lipid peroxidation in lungs is due to its capacity to generate great concentrations of free radicals caused by their reactivity towards lipids [23]. Similarly, in the study of Gao, *et al* [11], the persistent increase of MDA and therefore of the lipid peroxidation after exposition to BaP is in agreement with the literature for it is a well-known fact that the ROS take part in the BaP epoxidation and cause the increase in MDA excretion in the first days following the injection of BaP, which suggests here that the liver (more than the lung) is the organ where an important lipid peroxidation takes place. Furthermore, the decrease of the enzymatic antioxidant capacities can lead to an accumulation of the superoxide anion and an increase of lipid peroxidation, thus generating oxidative stress in the exposed organisms at relatively elevated concentrations of PAH.

IV. Conclusion

Results of the present study clearly demonstrated that exposition to BaP in acute conditions induces oxidative stress in both lung and liver in mice, and this was evidenced by a modification of the cellular oxidative status through a modulation of the activities of the major antioxidant enzymes and the GSH cellular content as well as an increase of lipid peroxidation. Oxidative stress represents an important part of BaP acute toxicity in mice and brings out the need of more advanced studies to develop innovative biomarkers and methods of risk assessment of environmental intoxications particularly for human health.

V. References

1. Ghorani-Azam, A.; Riahi-Zanjani, B.; Balali-Mood, M. Effects of air pollution on human health and practical measures for prevention in Iran. *Journal of Research in Medical Sciences*. 21 (2016) 65.
2. Kampa, M.; Castanas, E. Human health effects of air pollution. *Environmental Pollution* 151 (2008) 362-367.
3. Menzel, DB. The toxicity of air pollution in experimental animals and humans: the role of oxidative stress. *Toxicology Letters* 72 (1994) 269.
4. Rahman, I.; MacNee, W. Oxidative stress and regulation of glutathione in lung inflammation. *European Respiratory Journal* 16 (2000) 534.
5. Valko, M.; Leibfritz, D.; Moncol, J.; Cronin et al. Free radicals and antioxidants in normal physiological functions and human disease. *The international journal of Biochemistry & Cell Biology* 39 (2006) 44.
6. Ba, Q.; Li, J.; Huang, C.; et al. Effects of Benzo[a]pyrene Exposure on Human Hepatocellular Carcinoma Cell Angiogenesis, Metastasis, and NF-κB Signaling. *Environmental Health Perspectives* 123 (2015) 246-254.
7. Gelboin, HV. Benzo[a]pyrene metabolism, activation and carcinogenesis: role and regulation of mixed-function oxidases and related enzymes. *Physiological Reviews* 60 (1980) 1107-1166.
8. Rubin, H. Synergistic mechanisms in carcinogenesis by polycyclic aromatic hydrocarbons and by tobacco smoke: a bio-historical perspective with updates. *Carcinogenesis* 22 (2001) 1903-1930.
9. Umannová, L.; Machala, M.; Topinka, J et al. Benzo[a]pyrene and tumor necrosis factor-αcoordinately increase genotoxic damage and the production of pro inflammatory mediators in alveolar epithelial type II cells. *Toxicology Letters* 206 (2011) 121-129.
10. An, J.; Yin, L.; Shang, Y.; et al. The combined effects of BDE47 and BaP on oxidatively generated DNA damage in L02 cells and the possible molecular mechanism. *Mutation Research* 721 (2011) 192-8.
11. Gao, M.; Li, Y.; Long, J. Induction of oxidative stress and DNA damage in cervix in acute treatment with benzo[a]pyrene. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 719 (2011) 52-59.
12. Iqbal, M.; Sharma, SD.; Okazaki, Y et al. Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity. *Pharmacology and Toxicology* 92 (2003) 33-38.
13. Bradford, MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72 (1976) 248-254.
14. Ellman, GL. Tissue sulphydryl groups. *Archives of Biochemistry and Biophysics* 82 (1959) 70-77.
15. Claiborne, A. Catalase activity. In: R.A. Greenwald, ed. Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press (1985) 283-284.
16. Habig, WH.; Pabst, MJ.; Jakoby, WB. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. *Journal of Biological Chemistry* 249 (1974) 7130-7139.
17. Ohkawa, H.; Ohishi, N.; Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry* 95 (1979) 351-358.
18. Hegazy, AM.; Bakry, HH.; El-Shawarby, RM. Effects of benzo(a)pyrene on blood components, tumor markers, and oxidative status in mice. *Toxicological and Environmental Chemistry* 94 (2012) 136-145.
19. Budduluru, LN.; Kasala, ER.; Barua, CC et al. Antiproliferative and antioxidant potential of hesperetin against benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. *Chemico Biological Interactions* 242 (2015) 345-352.
20. Bonnard, N.; Brondeau, MT.; Clavel, T et al. Benzo(a)pyrène. *INRS* 144 (2007) 1-3.
21. Kumar, M.; Sharma, VL.; Sehgal, A et al. Protective Effects of Green and White Tea Against Benzo(a)pyrene Induced Oxidative Stress and DNA Damage in Murine Model. *Nutrition and Cancer* 64 (2012) 300-306.
22. Asha, S.; Girika, D. Benzo[a]pyrene induced liver and kidney cancer in swiss albino mice. *Pharmaceutical Research* 1 (2011) 22-27.

23. Selvendiran, K.; Banu, SM.; Sakthisekaran, D. Protective effect of piperine on benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice. *Clinica Chimica Acta* 350 (2004) 73-78.

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