Journal of New Technology and Materials (JNTM)
J. New Technol. Mater.
Vol. 09, N°02 (2019)10-16

OEB Univ. Publish. Co.

Beneficial effect of gallic acid on oxidative stress and chronic hepatotoxicity induced by carcinogenic benzopyrene in rats

Ilham Benchouieb[a], Hocine Rechreche[a*] and Mesbah Lahouel[b]

[a]*Molecular and Cellular Biology Laboratory, Mohamed Seddik Benyahia University, Department of molecular and cellular biology, Faculty of sciences Jijel, Algeria.*

[b]*Laboratory of Molecular Toxicology, Mohamed Seddik Benyahia University, Department of molecular and cellular biology, Faculty of sciences Jijel, Algeria.*

*Corresponding author, email: benilhamo@gmail.com

Received date: May 09, 2019 ; accepted date: Oct. 05, 2019

Abstract

Benzo[a]pyrene is an environmental pollutant, well known for its powerful genotoxic and carcinogenic effects in various organs. Gallic acid, a widely distributed phenolic acid, possesses strong antioxidant, anti-inflammatory and anti-carcinogenic activities. This study investigated the effects of gallic acid on chronic liver injury induced by benzo[a]pyrene in Wistar rats. The intoxicated group received an intraperitoneally single dose of benzo[a]pyrene at 100 mg/kg, while the treated animals were intraperitoneally injected with benzo[a]pyrene at 100 mg/kg and then they orally received gallic acid at 100 mg/kg per day for five consecutive days. The liver toxicity was assessed by evaluation of the serum hepatic markers (alanine aminotransferase and aspartate aminotransferase), the enzymatic and nonenzymatic oxidative stress indicators and the histopathological changes. The benzo[a]pyrene administration increased the levels of malondialdehyde with a concomitant decrease in superoxide dismutase, glutathione-s-transferase and glutathione. Parralelly, liver histological alterations were observed. The animals treatment with gallic acid restored the alterations in liver tissues, decreased lipid peroxidation and serum marker enzyme activity and significantly improved the overall antioxidant capacity, suggesting that gallic acid was responsible of a beneficial effects against oxidative stress and hepatotoxicity induced by benzo[a]pyrene in rat.
Keywords: carcinogen; benzopyrene; gallic acid; oxidative stress; hepatotoxicity; rat.

1. Introduction

Benzo[a]pyrene or B(a)P is a widespread and ubiquitous environmental pollutant who belongs to the polycyclic aromatic hydrocarbon (PAH) group. PAH are found in incineration by-products from industries, exhaust fumes of vehicles, tabacco smoke and charcoal-grilled food, and also comes from natural sources such as forest fires and volcanoes [1, 2]. Therefore, the human body is exposed to this pollutant via various sources: polluted environment and contaminated food representing the most important paths [3]. B(a)P is the most extensively studied PAH, a genotoxicant model used particularly in experimental studies on animals [4].It is a powerful mutagenic and carcinogenic agent which has been shown to induce gene mutations, chromosomal aberrations and other types of genotoxic effects in various experimental systems. It has been suggested that B(a)P may induce cancers of lungs, stomach, breast, urinary bladder, prostate and skin [5]. The metabolisation of B(a)P leads to the production of chionone derivatives including B(a)P-6,12-dione, B(a)P-3,6-dione and B(a)P-1,6-dione, via monoelectron oxidation [6]. The result is the generation of oxygen free radicals responsible for the oxidation of cellular macromolecules such as DNA, RNA, proteins, lipids and carbohydrates leading to structural damage, serious alterations of cellular functions and hence, promoting inflammation, necrosis, fibrosis and even carcinogenesis [7, 8].

To deal with these detrimental and malignant effects, researchers have recently focused their attention on the dietary intake of medicinal plants, fruits and vegetables that has been proven in the effective protection against cancer. Such favorable effects are due to the antioxidant activity of phenolic products, their strong capacity to scavenge free radicals and their redox properties [9]. Gallic acid (3, 4, 5-trihydroxybenzoicacid) or GA is a natural polyphenol found in several plants species such as gallnuts, sumac, tea leaves, apple-peels, grapes, bananas, lemon, mango and blackberries [8,10]. Like other phenolic acids, GA possesses an important pharmacological properties, including, anti-allergic, anti-inflammatory, anti-tumoral, antiviral, antifungal and antibacterial [11, 12]. GA-anticancer effects involved the cell death of numerous tumor cell lines by exerting cytotoxicity and antimutagenic properties [9]. On the other hand, It has been reported that GA showed strong antioxidant activities through displaying free radical scavenging and reinstating the antioxidant enzymes activities, such as catalase and glutathione peroxidase [13, 14].

The B(a)P administration may lead to produce tumors in animal models for various organs including the gastro intestinal tract, mammary glands and liver [15]. Moreover, epidemiological studies suggested the association between the PAH-DNA adducts and the elevated risk of

hepatocellular carcinoma. Indeed, the environmental exposure to high levels of B(a)P increases the risk of this cancer, sugesting that B(a)P may be a cause of human hepatocellular carcinoma [16]. However, B(a)P does not cause cancers until it is metabolized to toxic intermediates by cytochrome P450 enzymes. Liver tissues have the highest capacity for such biotransformation, making it sensitive to B(a)P exposure [17]. Consequently, in this study, we aimed to determine the effect of GA on B(a)P-induced oxidative stress and liver injury in Wistar rats, as there are at this time no scientific reports related to GA action against B(a)P hepatotoxicity and only few studies described the long term effects of this pollutant in rats.

2. Materials and methods

2.1. Chemicals

B(a)P (purity <96% HPLC) and GA were respectivelly purchased from Sigma Aldrich Co (St. Louis, USA) and Alfa Aesar, a Johnson Matthey Company (Karlsruhe, Germany).

2.2. Animals and husbandry

Wistar albino male rats weighing 180-200g were purchased from Pasteur Institute (Algiers, Algeria). The rats were 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.

2.3. Experimental design

Our study was conducted in collaboration with the study of Lariche et al [18] where, Wistar rats were given a single injection of benzo[a]pyrene at 100 mg /kg intraperitoneally in order to induce lung cancer. B(a)P and GA were respectively dissolved in sunflower oil and 0.9% NaCl [3,11]. Their doses were selected according to the literature [1, 8]. Twenty-four male Wistar rats were divided into four groups of 6 rats each: the group 1 (control group) received an i.p. injection of 1ml sunflower oil and after 24 weeks, received 1ml of NaCl 0.9% by oral gavage, per day for five consecutive days. The group 2 (intoxicated group) received a single i.p. injection of 100 mg/kg of B(a)P and also received, after 14 weeks, 1ml of NaCl 0.9% by oral gavage, per day for five consecutive days. The group 3 (The treated group) received a single i.p. injection of B(a)P and after 24 weeks, received orally 100 mg/kg of GA per day, for five consecutive days. Group 4 (The GA-group) received orally 100 mg/kg of GA per day, for five consecutive days and also received an i.p. injection of 1ml sunflower oil, at the start of the experiment.

Forty-eight hours following the last injection of GA, blood samples were collected by ocular vein puncture in heparinized tubes and serums were separated by centrifugation at 3000 r.p.m for 15 minutes. Rats were then sacrificed by cervical dislocation and their liver was quickly removed, washed in an ice-cold saline solution and stored in -80°C for different means of measurement.

2.4. Biochemical examination of liver tissue

The frozen liver tissue samples were quickly weighed (0.5g) before being homogenized in ice-cold 3 volumes of 100 mM potassium phosphate buffer (pH=7.4) containing 1.15M KCl. The homogenates were centifuged at 4000×g for 15 min, at 4°C, then the supernatant was centrifuged at 10,000×g for 30 min at 4°C [19]. The supernatants were separated and used for enzyme activity assays. Total protein concentration was estimated by the technique of Bradford [20].

2.4.1. Total Glutathione Estimation

The hepatic glutathione (GSH) content was estimated by the method of Ellman [21]. 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.

2.4.2. Antioxidant enzymes activities measurement

The Catalase (CAT) activity was measured as described by Claiborne [22], 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 superoxide dismutase (SOD) activity was assayed by evaluating its ability to inhibit the nitroblue tetrazolium (NBT) photochemical reduction at 560 nm [23]. The reaction mixture was composed of sodium pyrophosphate buffer (pH 7.8, 50mM), EDTA (0.66 mM), nitroblue tetrazolium (33 µM), riboflavine (0.0033 mM) and methionine (10 mM). The samples were submited to the luminescent lamps for 10 min, and then the SOD activity was obtained by measuring the optical extinction at 560 nm. The glutathione-S-transferase (GST) activity was determined as described by Habig *et al* [24]. The reaction mixture (0.1 M PBS (pH 6.5) and 20 mM 1-chloro-2, 6-dinitrobenzene) 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.

2.4.3. Measurement of lipid peroxidation level

Lipid peroxidation (LPO) was estimated by using a modified method of Ohkawa [25], 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 4ml 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.

2.5. Measurement of serum transaminase activities

The enzymatic activities of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT)

were measured on serum using automate COBAS INTEGRA® 400 plus, from Roche Diagnostics (Basel, Switzerland).

2.6. Histopathological examination of liver sections

A small portion of fixed liver tissues in 10% neutral buffered formalin from the experimental animals were washed overnight, dehydrated through graded alcohols, and embedded in paraffin wax. Serial sections of 5μm thickness were stained with haematoxylin and eosin (H&E) for histological examination. All sections from the experimental animals were observed and photomicrographed under light microscope (Olympus BX-51, Tokyo, Japan).

2.7. 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.

2. Results and discussion

3.1. Evaluation of liver oxidative stress

The changes in hepatic GSH content of each group are depicted in Figure 1. In comparison with the basal value of the control group, the oxidative stress caused by B(a)P dramatically depleted the intracellular GSH ($p< 0.001$) while post-treatment with GA significantly altered the reduction of GSH ($p< 0.05$). In fact, the glutathion decrease in the intoxicated group was estimated at 65%, versus a total recovery of GSH content in the treated group.

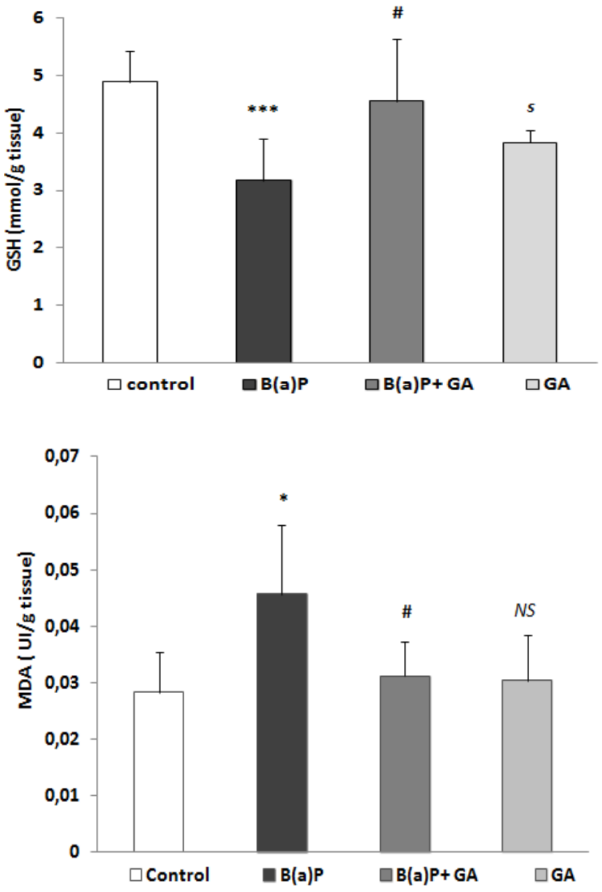

Figure 1. Level of GSH and MDA in liver tissue of rats, 14 weeks after intraperitoneal injection of 100mg/kg of B(a)P and treatment by GA at 100mg/kg for five consecutive days. Data are expressed as mean ±SD (n=6). * significantly different from the control group at $P\leq0.05$; *** significantly different from control group at $P\leq0.001$; # significantly different from B(a)P treated alone group at $P\leq0.05$; *S* significantly different from control group $P\leq0.05$; NS: not significant, from the control group.

The activity of GST dramaticaly decreased by 58% in the intoxicated group compared with the control group ($p< 0.001$) while the treated group by GA presented a highly significant activity of 63.19% compared to the intoxicated group ($p< 0.01$) (Figure 2).

Table1. Effect of GA on serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels (IU/L) in B(a)P intoxication. A student test was used to measure the difference in serum AST and ALT among the four animal groups. Values are mean ± SD (n=6); ** significantly different from the control group at $P\leq0.01$; # significantly different from the intoxicaed group at $P\leq0.05$; *NS:* not significant from the control group.

	Control	B(a)P	B(a)P+GA	GA
ALT	57.3±15.3	107.5±14.9 **	68±10.8 #	73.2±10.7 NS
AST	111.8±17.2	178.2±14.8 **	106.3±18.5 #	112.5±13.5 NS

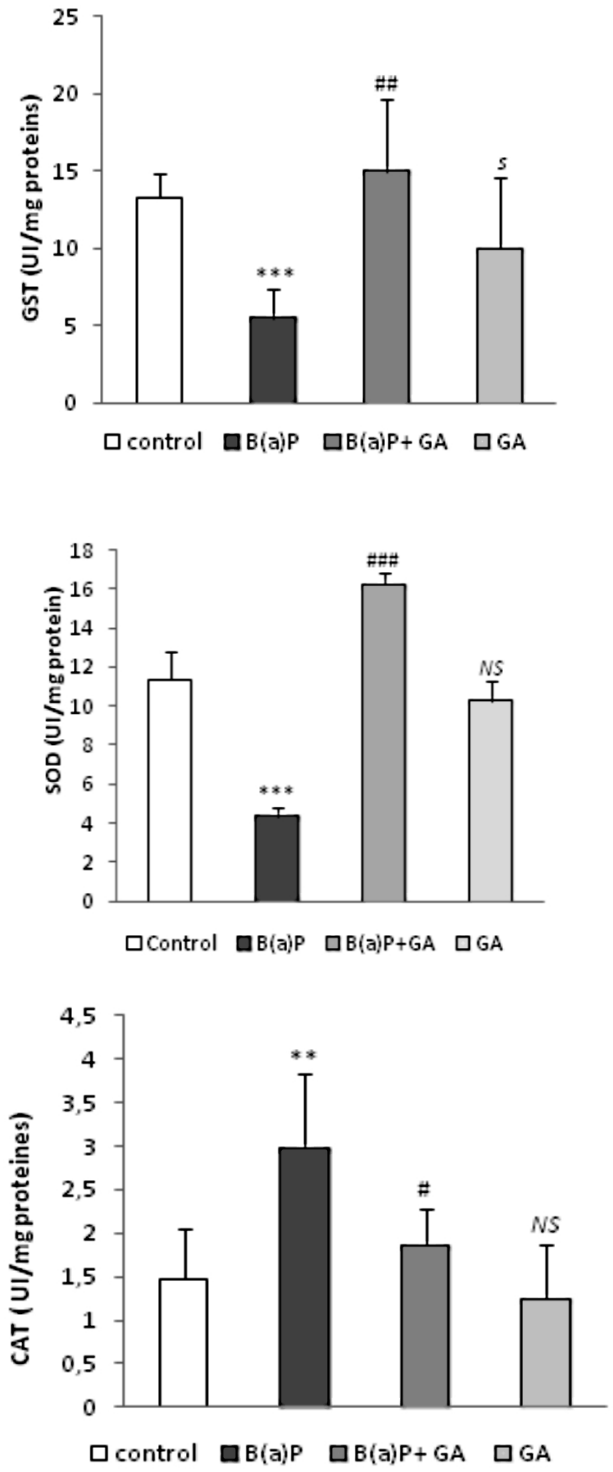

Figure 2. Effects of B(a)P and GA administration on the enzymatic activity of hepatic GST, SOD and CAT in the four groups of rats, the control group receiving sunflower oil and 0.9%NaCl (control), the intoxicated group receiving *IP* 100mg/kg of B(a)P (B(a)P), the intoxicated and treated by 100mg/kg of GA goup (B(a)P+GA) and GA group (GA). Data are expressed as mean ± SD. *** significantly different from control group at $P \leq 0.001$; ** significantly different from control group at $P \leq 0.01$; # significantly different from B(a)P-intoxicated group at $P \leq 0.05$; ## significantly different from B(a)P intoxicated group at $P \leq 0.01$; ### significantly different from B(a)P intoxicated group at $P \leq 0.001$; *S* significantly different from the control group $P \leq 0.05$; NS: not significant, from the control group.

SOD activity results showed the most obvious variations. The enzymatic activity of B(a)P-intoxicated rats clearly decreased from a normal value of 11.33 UI/mg protein in the control rats down to 4.35 UI/mg protein ($p < 0.001$) (Figure 2) whereas the GA-treated rats demonstrated a higher increase in SOD activity than that of the control rats ($p < 0.001$). The GA group showed no significant difference compared to the control group.

The intoxicated group demonstrated an important increase in CAT activity compared to the control ($p < 0.01$) while it was back to normalcy in the group post-treated by GA ($p < 0.05$) and no significant difference was observed between the control and the GA groups (Figure 2).

Figure 1 shows that chronic intoxication with B(a)P caused the highest liver MDA level of all the groups ($p < 0.05$), however, the MDA level was significantly lower in the rats post-treated with GA than those not treated and apparently had the MDA level returned to normalcy ($p < 0.05$). The GA group presented no significant difference compared with the control group.

3.2. Statement of liver function

As shown in Table1, treatment of rats with B(a)P induces a highly significant increase in the plasmatic levels of both transaminases (ALT, AST); the rats group receiving B(a)P intraperitoneally at the dose of 100 mg/kg showed an increased value of ALT in the intoxicated group ($p < 0.01$) while a significantly decreased value of ALT was recorded respectively in the intoxicated group post-treated with GA and the GA group ($p < 0.05$). The same observations were registered in AST values with a significant elevation in the intoxicated group ($p < 0.01$) as well as a significant decrease in the one treated with GA ($p < 0.05$).

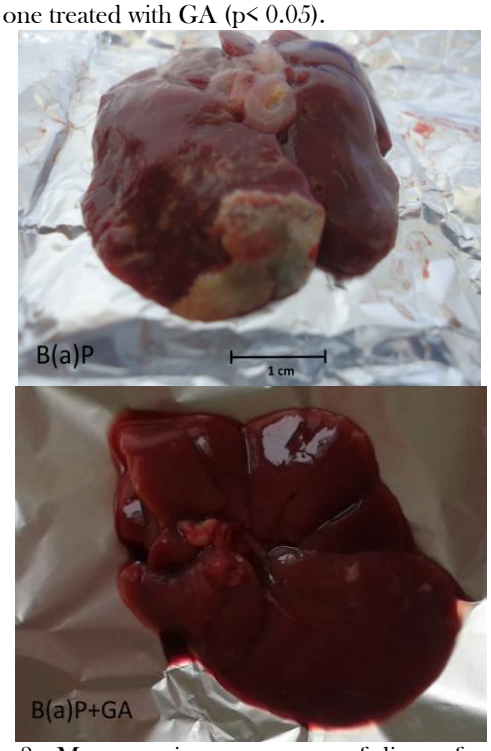

Figure 3. Macroscopic appearance of livers from the intoxicated rats receiving 100mg/kg of B(a)P revealing

yellowish white irregular-shaped area in addition to adjacent hepatic lesions (B(a)P) and the intoxicated rats treated by 100mg/kg of GA for five consecutive days following the chronic intoxication, revealing white spots, a reddish brown colour and no apparent lesions (B(a)P+GA).

Macroscopic analyses of livers from the B(a)P-intoxicated and the GA-treated rats presented important variations. In the B(a)P-intoxicated group, the hepatic lesion was explicit, showing yellowish-white coloration and hardening with a diameter between 1.0 and 1.2 cm, there were also indications of adjacent hepatic lesions with a slightly more extensive area (1.2 to 1.5 cm). These lesions were reddish-white in coloration, cystic in appearance and with imprecise borders (although limited). The intoxicated rats post-treated with GA ilustrated no apparent lesions but the presence of white spots and a reddish-brown coloration.

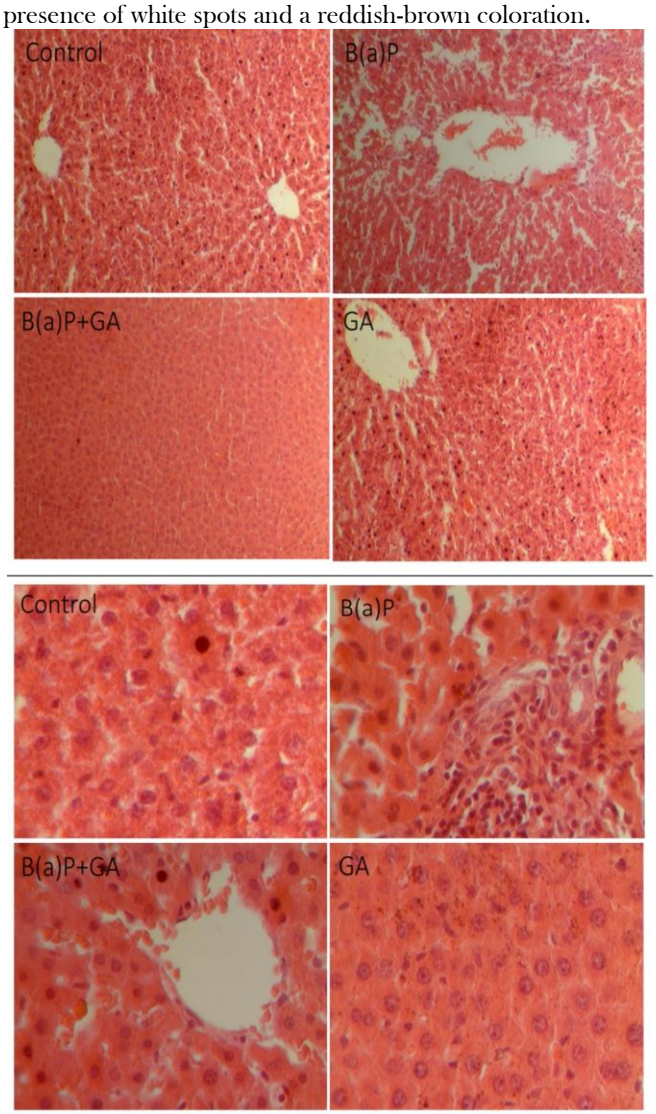

Figure 4. Liver sections of histopathological evaluation of the effect of GA on B(a)P-induced hepatotoxicity. Figures are representative from the four animal groups : the control group, receiving sunflower oil and 0.9% NaCl (control); the intoxicated group, receiving 100 mg/kg of B(a)P (B(a)P); the intoxicated and treated by 100mg/kg of GA group (B(a)P+GA) and the GA-group, receiving 100mg/kg of GA alone (GA), at 10× (a) and 40× magnification (b).

H&E staining and microscopic examination (10×, 40×) of liver sections from the four different groups (Figure 4) explain that liver sections from the control and GA-groups showed normal morphological and histological features, with normal hepatocytes without any inflammation or necrosis. Liver sections from rats intoxicated with B(a)P showed obvious infiltration of inflammatory cells in the periportal tract, hemorrage, hydropnic degeneration, pycnosis, sinusoids dilatation, hepatocytes degeneration along with necrotic damage. However, livers from the group of rats treated with GA after B(a)P chronic intoxication showed markedly improved histological features as well as a reduced infiltration of inflammatory cells and necrotic damage.

The main etiology of lung cancer beside intrinsic factors (such as heredity) is smoking, air pollution and occupation factors. Consequently, B(a)P, as a carcinogen found in cigarettes is used for inducing lung cancer in animal models. In fact, in a recent study [26], rats were exposed to B(a)P and had developed lung cancer, at the end of four months, in a previous study [27] the production of an experimental model of pulmonary carcinogenesis in Wistar rats, was realised by giving a single intrapulmonary instillation of B(a)P at doses of 10 and 20 mg/kg and the animals were killed after 8, 10, 12 and 14 weeks and reduction in the antioxidant defense mechanism is one of the major reasons behind BaP-induced carcinogenicity, is related to a reduction in the activities of anti-oxidative enzymes including SOD, catalase and GSH which are responsible for ROS scavenging in synergy.

As B(a)P carcinogenicity in organs such as lungs and skin is no longer to be proven, in the present investigation we focused on liver which is primarily exposed to this xenobiotic, in order to highlight remote effects and further pathways of a unique administration of this carcinogenic coumpound. In this purpose, we examined the changes in toxic biomarkers and oxidative stress indicators through the activities of ALT, AST, CAT, SOD, GST, the levels of GSH and LPO, as well as the macroscopic and the histopathologic aspects of the rat liver. The results obtained after B(a)P intoxication generally indicate a reduction of the antioxidative defense system capacities, since the most important antioxidant enzyme activities decreased.

GSH is a key antioxidant that is determinant in the regulation of redox cellular state, in the protection against ROS, regeneration of vitamine C and E and thus in the prevention of oxidative stress and detoxification [28]. The changes in hepatic reduced-GSH content demonstrated that the oxidative stress caused by B(a)P dramatically depleted intracellular GSH while post-treatment with GA significantly altered the reduction of GSH. Indeed, the difference was obvious between the glutathione decrease in B(a)P intoxication and the almost total recovery of GSH content in the GA-treated group. GST, which catalyses the conjugation of reduced glutathione (GSH) with xenobiotics allowing their excretion [28, 29], demonstrated a clearly significant low activity along with the GSH low content. This may be the result of their excessive use in the neutralization of important amounts of free radicals generated in this situation and the

inhibition of enzymes involved in the synthesis and the regeneration of GSH, such as glutathione peroxidase and glutathione reductase, facts that would induce oxidative stress and cause severe damage to liver cells [29]. GSH level also significantly increased in the intoxicated group post-treated by GA, in line with the experiments of Navabi *et al* [10] who brought to light the enhancement of GSH/GSSG ratio and the supression of reactive species generation by GA. SOD is responsible for dismutating superoxide anion into H_2O_2 and molecular oxygen to ensure the defense against free radicals [30]. Its reduction in the present study reflects not only the inability to eliminate superoxide anion but also, a susceptibility to oxidative diseases.

Suprisingly, CAT which is responsible for H_2O_2 detoxification into water displayed a notably increased activity in the intoxicated group. Then, it gets back to a normal value in the intoxicated group treated by GA. This can be explained by high concentrations of H_2O_2 produced not only by SOD but also by peroxisomal and microsomal enzymes [7] and B(a)P activation pathways. GPx enzyme, in charge of hydrogen peroxide elimination at low intracellular levels – case of low intensity oxidative stress – soon reaches saturation. CAT is therefore more efficient because it cannot be saturated even at very high concentrations of H_2O_2.

Similar to other studies of Ma et al and Reckziegel et al [8, 31], treatment with GA seems to reverse the results above by increasing the enzymatic activity of GST and SOD and getting CAT back to normal value, thanks to its powerful antioxidant capacity and ability to scavenge free radicals. This can be explained by a structure-activity relationship as this phenolic acid possesses three hydroxyl groups at para position to the carboxylic group (see Figure 1), a very effective position to form stable phenoxy radicals by donating a proton from their O-H.

Oxidative damage can also be measured through lipid peroxidation [28] and its end-product MDA. Even if LPO is a normal physiological process mediated by non enzymatic and free radicals chain reaction, its level is raised by excessive oxidative stress [6]. The chronic intoxication by B(a)P induces an important lipid peroxidation indicating cell membrane damage and intercellular membrane disruption, as a result of high rate generated reactive oxygen and nitrogen species as well as decreased antioxidants [32]. Treatment with GA of B(a)P intoxicated rats reduces significantly the MDA level, due to free radical scavenging activity of GA and, in so doing, protects membrane structure and function.

One sign of liver cells injury is the release into the bloodstream of intracellular enzymes like transaminases. The elevation of ALT and AST activities after B(a)P administration explains the leakage of functional hepatocytes and membrane integrity, whereas the reduced values of serum transaminases in the intoxicated rats treated with GA reflect the restoration of hepatocytes physiological integrity [33].

Finally, the obvious variations in liver macroscopy and histology among the different groups revealed serious histological changes and abnormalities predicting a possible hepatocarcinogenesis in B(a)P intoxicated rats whereas the treatment with GA showed, in correlation with previous studies of [11, 13] that this antioxidant had the ability to improve the hepatic tissue towards a physiological structure with normal features, i.e, restored cellular integrity of hepatocytes, attenuated cell infiltration, sinusoidal dilatation and reduced necrosis for a well preserved histological architecture.

4. Conclusion

Data of the present study demonstrate clearly that B(a)P has deleterious effects on rats liver. GA, with a curative potential, has a great capacity to counter the noxious carcinogenic action of B(a)P by checking the spread of free radicals with a powerful scavenging action, restoring the antioxidant status, inhibiting lipid peroxidation and improving the hepatic physiological function, thus confirming its "super-antioxidant" appellation. These results are an enticement to investigate in future studies, the possible role of GA in the protection against the various toxic effects arising from the activation of these harmful compounds through other signaling pathways

Acknowledgments

This work was supported by a grant allocated by the "Ministère de l'Enseignement Supérieur et de la Recherche Scientifique" of Algeria (Project code: F01720120001).

References

[1] C. R. Saunders, D. C. Shockley, M. E. Knuckles, "Behavioral effects induced by acute exposure to benzo(a)pyrene in F-344 rats", Neurotox. Res, 3, (2001) 557-579.

[2] S. Uno, K. Sakurai, D. W. Nebert, M. Makishima, "Protective role of cytochrome P450 1A1 (CYP1A1) against benzo[a]pyrene-induced toxicity in mouse aorta", Toxicology, 316, (2014) 34-42.

[3] E. M. Ciałowicz, Z. Jethon, J. Magdalan, "Effects of melatonin on lipid peroxidation and antioxidative enzyme activities in the liver, kidneys and brain of rats administered with benzo(a)pyrene", Exp. Toxicol. Pathol, 63, (2011) 97-103.

[4]. P. Hodek, P. Fousova, E. Brabencova, "Effect of dihydromyricetin on benzo[a]pyrene activation in rats", Neuroendocrinol. Lett, 35, (2014) 158-168.

[5] Q. Ba, J. Li, C. Huang, "Effects of benzo[a]pyrene exposure on human hepatocellular carcinoma cell angiogenesis, metastasis, and NF-κB signaling", Environ. Health. Perspect, 123, (2015) 246-254.

[6] A. M. Hegazy, H. H. Bakry, R. M. El-Shawarby, "Effects of benzo(a)pyrene on blood components, tumor markers, and oxidative status in mice", Toxicol. Environ. Chem, 94, (2012) 136-145.

[7] F. Henkler, J. Brinkmann, A. Luch, "The role of oxidative stress in carcinogenesis induced by metals and xenobiotics", Cancers, 2, (2010) 376-396.

[8] S. Ma, L. Lv, Q. Lu, "Gallic acid attenuates dimethylnitrosamine-induced acute liver injury in mice through Nrf2-mediated induction of heme oxygenase-1

and glutathione-s-transferase alpha 3", Med. Chem, 4, (2014) 663-669.

[9] S. Jagan, G. Ramakrishnan, P. Anandakumar, "Antiproliferative potential of gallic acid against diethylnitrosamine-induced rat hepatocellular carcinoma" Mol. Cell. Biochem, 319, (2008) 51-59.

[10] S. F. Nabavi, S. Habtemariam, M. Jafari, "Protective role of gallic acid on sodium fluoride induced oxidative stress in rat brain. Bull. Environ", Contam. Toxicol, 89, (2012) 73-77.

[11] M. Canbek, M. C. Ustüner, S. Kabay, " The effect of gallic acid on kidney and liver after experimental renal ischemia/reperfusion injury in the rats", Afr. J. Pharm. Pharmacol, 5, (2011) 1027-1033.

[12] S. M. Nabavi, S. Habtemariam, S. F. Nabavi, "Protective effect of gallic acid isolated from Peltiphyllum peltatum against sodium fluoride-induced oxidative stress in rat's kidney", Mol. Cell. Biochem, 372, (2013) 233-239.

[13] V. V. Padma, P. Sowmya, T. A. Felix, "Protective effect of gallic acid against lindane induced toxicity in experimental rats", Food. Chem. Toxicol, 49, (2011) 991-998.

[14] S. I. Abdelwahab, "Protective mechanism of gallic acid and its novel derivative against ethanol-induced 2 gastric ulcerogenesis: Involvement of immunomodulation markers, Hsp70 and 3 Bcl-2-associated X protein", Int. Immunopharmacol, 16, (2013) 296-305.

[15] D. Benford, M. Dinovi, R. W. Setzer, "Application of the margin-of-exposure (MoE) approach to substances in food that are genotoxic and carcinogenic e.g.: benzo[a]pyrene and poly cyclic aromatic hydrocarbons", Food. Chem. Toxicol, 48, (2010) 42-48.

[16] Y. Su, B. Zhao, F. Guo, Z. Bin, Y. Yang, S. Liu, "Interaction of benzo[a]pyrene with other risk factors in hepatocellular carcinoma: a casecontrol study in Xiamen", China. Ann. Epidemiol, 24, (2014) 98–103.

[17] Q. Ba, J. Li, C. Huang, H. Qiu, J. Li, R. Chu, "Effects of Benzo[a]pyrene Exposure on Human Hepatocellular Carcinoma Cell Angiogenesis, Metastasis, and NF-κB Signaling. Environ", Health. Perspect, 123, (2015) 246-254.

[18] N. Lariche, M. Lahouel, L. Benguedouar, "Ferulenol, a Sesquiterpene Coumarin, Induce Apoptosis via Mitochondrial Dysregulation in Lung Cancer Induced by Benzo[a]pyrene: Involvement of Bcl2 Protein", Anticancer. Agents. Med. Chem, 17, (2017) 1-6.

[19] M. Iqbal, S. D. Sharma, Y. Okazaki, "Dietary supplementation of curcumin enhances antioxidant and phase II metabolizing enzymes in ddY male mice: possible role in protection against chemical carcinogenesis and toxicity", Pharmacol. Toxicol, 92, (2003) 33-38.

[20] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding", Anal. Biochem, 72, (1976) 248-254.

[21] G. L. Ellman, "Tissue sulphydryl groups", Arch. Biochem. Biophys, 82, (1959) 70-77.

[22] A. Claiborne, "Catalase activity. In R.A. Greenwald (eds.), *Handbook of Methods for Oxygen Radical Research.* Boca Raton: CRC Press, 1985, pp. 283-284.

[23] C. Beauchamp, I. Fridovich, "Superoxide dismutase: improved assays and an assay applicable to acrylamide gels", Anal. Biochem, 44, (1971) 276-287.

[24] W. H. Habig, M. J. Pabst, W. B. Jakoby, "Glutathione S-transferases: The first enzymatic step in mercapturic acid formation", J. Biol. Chem, 249, (1974) 7130-7139.

[25] H. Ohkawa, N. Ohishi, K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction", Anal. Biochem, 95, (1979) 351-358.

[26] S. Sikdar, A. Mukherjee, A. R. Khuda-Bukhsh, "Ethanolic Extract of Marsdenia condurango Ameliorates Benzo[a]pyrene-induced Lung Cancer of Rats", J. Pharmacopuncture, 17, (2014) 007-017.

[27] B. A. K. da Silva, I. S. Silva, D. M. Pereira, "Experimental model of pulmonary carcinogenesis in Wistarrats", Acta Cir Bras, 22, (2007) 16-20.

[28] M. Gao, Y. Li, J. Long, "Induction of oxidative stress and DNA damage in cervix in acute treatment with benzo[a]pyrene", Mutat. Res. Genet. Toxicol. Environ. Mutagen, 719, (2011) 52-59.

[29] S. I. Gum, S. J. Jo, S. H. Ahn, "The potent protective effect of wild ginseng (Panax ginseng C.A. Meyer) against benzo[a]pyrene-induced toxicity through metabolic regulation of CYP1A1 and GSTs", J. Ethnopharmacol, 112, (2007) 568-576.

[30] K. P. Reddy, P. Madhu, P. S. Reddy, "Induction of oxidative stress by benzo(a)pyrene in the epididymis of Wistar rats", Toxicol. Environ. Chem, 97, (2015) 1226-1235.

[31] P. Reckziegel, V. T. Dias, D. M. Benvegnú, "Antioxidant protection of gallic acid against toxicity induced by Pb in blood, liver and kidney of rats", Toxicol. Rep, 3, (2016) 351-356.

[32] M. Repetto, J. Semprine, A. Boveris, "Lipid peroxidation: Chemical mechanism, biological implications and analytical determination. In A. Catala (eds.), Lipid peroxidation", Rijeka: InTech, 2012, pp. 3-33.

[33] E. T. Olayinka, A. Ore, O. S. Ola, O. A. Adeyemo, "Ameliorative effect of gallic acid on cyclophosphamide-induced oxidative injury and hepatic dysfunction in rats", Med. Sci, 3, (2015) 78-92.