African Scientist Vol. 22, No. 4 December 31, 2021
Printed in Nigeria

1595-6881/2021 $10.00 + 0.00

http://www.niseb.org/afs

AFS2021014/22401

Modulatory Effects of Curcumin on Antioxidant Status of Benzo[a]pyrene Treated Albino Rats

Kolade, O.Y.[1]* and Oladiji, T.A.[2]

[1]Nigerian Institute for Oceanography and Marine Research P. M. B. 12729, Victoria Island, Lagos.
[2]Department Of Biochemistry, Faculty of life Sciences, University Of Ilorin, Ilorin, Kwara State

*Corresponding author; Email: kemmystick2006@gmail.com Tel: +234 (0) 803 074 9318

(Received September 26, 2021; Accepted in revised form November 29, 2021)

ABSTRACT: This study examined the modulatory effects of Curcumin on the antioxidant status of benzo[a]pyrene-treated albino rats. Thirty albino rats were divided into five treatment groups (n=6) namely: control, benzo**[**a]pyrene only (1 mg/kg), benzo**[a]**pyrene (1 mg/kg) + Curcumin (50 mg/kg), benzo**[a]**pyrene (1 mg/kg) + Curcumin (100 mg/kg), Benzo**[a]**pyrene (1 mg/kg) + Curcumin (200 mg/kg) for 6 weeks. Rats were administered the drug (benzo**[a]**pyrene and Curcumin) orally, thrice a week**.** Activities of antioxidant enzymes [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and concentrations of reduced glutathione (GSH)] and malondialdehyde (MDA) were estimated. Benzo[a]pyrene treatment only on rats induced significant increases (P<0.05) on malondialdehyde levels, and significant (P<0.05) decreases in activities of SOD, CAT, GSH and GPx. Curcumin administration helped to reduce MDA concentrations (P<0.05) in the rats earlier treated with benzo[a]pyrene and varying concentrations of Curcumin. Meanwhile activities of SOD and GSH of rats treated with 200 mg of Curcumin were significantly increased (P<0.05) compared to control group. The activity of GPx in rats treated with 200 mg of Curcumin, compared favorably with that of control. Thus, higher dose of Curcumin administered on the rats seemed to improve the activities of the antioxidant enzymes. Results of the study indicates that Curcumin treatment helped to modulate B[a]P-induced toxicities on the antioxidant status of the rats.

Keywords: Curcumin, Benzo[a]pyrene, Antioxidant enzymes, Antioxidant status

Introduction

Benzo[a]pyrene (BaP), a pentacyclic structured polycyclic aromatic hydrocarbon (Celik, 2019), is a potent carcinogen, considered to be an indicator of the carcinogenic potency of the polycyclic aromatic hydrocarbons (PAH) by World Health Organization (WHO) (Delgado-Saborit *et al*., 2010). It occurs in coal tar, in automobile exhaust fumes (particularly diesel engines) and in all fumes from organic materials (Aygün and Kabadayi, 2005). It is generated during food processing methods such as broiling, frying, and smoking. Following its absorption, BaP is metabolized to reactive intermediates such as B[a]P-7,8-diol-9,10-epoxide (BPDE), its ultimate metabolite (Gelboin1980), by cytochrome P450 (CYP) enzymes. This metabolism of BaP leads to production of reactive oxygen species (ROS) (Briede *et al*., 2004), bringing about oxidative stress (Briede *et al*., 2004; Costa *et al*., 2010), which then leads to oxidative DNA damage, lipid peroxidation, and the oxidation of several proteins, ultimately causing cytotoxicity and cell death (Ranjit *et al*., 2018).
Oxidative stress occurs when there is excessive free radical production and/or low antioxidant defense, and results in chemical alterations of biomolecules causing structural and functional modification (Chiarugi, 2003). The antioxidant defense system help to act upon and eradicate free radicals generated under normal body functioning conditions (Dutta *et al*., 2010). Various natural antioxidants are able to scavenge free radicals and prevent oxidative damage (Aladag *et al*., 2009; Harynua *et al*., 2017). Since free radicals are continuously generated *in vivo*, there are certain protective antioxidant enzymes such as superoxide dismutase, catalase,

glutathione-S-transferase, glutathione peroxidase and reduced glutathione which help to deal with these toxic substances (Tong *et al*., 2004).
Plant products tend to exert their protective potentials by scavenging free radicals and modulating carcinogen detoxification and antioxidant defense system. Naturally occurring compounds have been used to minimize B[a]P-induced toxicity (Sak, 2014; Adeneye, 2008). Amongst them is a polyphenol known as curcumin. Curcumin, the active ingredient of *Curcuma longa*, is widely utilized as a spice as well as coloring agent in some herbal formulation and food preparations. It has been documented as an inhibitor of inflammation, and oxidative stress (Wang *et al*., 2012) while playing a vital role in the treatment of various cancers (Rahmani *et al*., 2018; Rahmani *et al*., 2014). Thus the present study was undertaken to evaluate the modulatory effects of Curcumin on antioxidant status during benzo(a)pyrene induced toxicity in Wistar rats.

Materials and Methods

*Materials**:*** This study was conducted (with 30 mature Wistar male rats) in the laboratory of the Department of Biochemistry, University Of Ilorin, Ilorin, Kwara State, Nigeria. The animals weighing 120 to 150 g, were divided equally into 5 groups with six rats in each group and were allowed to get used to conditions of the laboratory, patterns of feeding and procedures of handling for one week, before the startup of the experiment. The animals had free access to standard animal diet and water *ad libitum*.
Reagents: Benzo[a]pyrene and Curcumin used in the study, were purchased from Sigma-Aldrich Chemicals Company (St. Louis, MO, U.S.A.).
Treatment schedule: The thirty male albino rats (*Rattus norvegicus*) were segregated into five different groups:
Group 1 (Control): Received rat chow and tap water only.
Group II: Received 1 mg/kg of B[a]P three times a week for 6 weeks.
Group III: Received 1 mg/kg of B[a]P + 50 mg/kg of Curcumin every other day for 6 weeks.
Group IV: Received 1 mg/kg of B[a]P + 100 mg/kg of Curcumin every other day for 6 weeks.
Group V: Received 1 mg/kg of B(a)P + 200 mg/kg of Curcumin every other day for 6 weeks.
Ethical Clearance: Ethical clearance for the study was obtained from University of Ilorin Ethical Review Committee with the UERC Approval number UERC/ASN/2018/1329.
Methods: After the experiment was completed, animals were treated with diethyl ether and sacrificed. Blood was taken via the jugular vein with a sharp sterile blade and centrifuged at 1500 rpm for 10 min to obtain serum. Livers of rats were removed, homogenized respectively in 0.25 M sucrose solution and centrifuged at 4,500 rpm. Resulting supernatants obtained were used for the various biochemical measurements.
Biochemical determinations: Lipid peroxidation assay was carried out according to the method of Wills (1966). Catalase activity was determined using the method of Luck (1965). Superoxide dismutase (SOD) activity was estimated by using the method of Kono (1978). The estimation of reduced glutathione (GSH) was determined by using the method of Ellman (1959).

Results

Effects of curcumin administration on the activities of catalase in benzo[a]pyrene treated albino rats:-The activities of Catalase (CAT) in the animals were significantly ($P<0.05$) reduced following the administration of 1 mg/kg body weight of B[a]P only on the rats (Figure 1). Significant increases in the activities of this enzyme was observed following the administration of Curcumin at the different doses on the B[a]P treated rats.

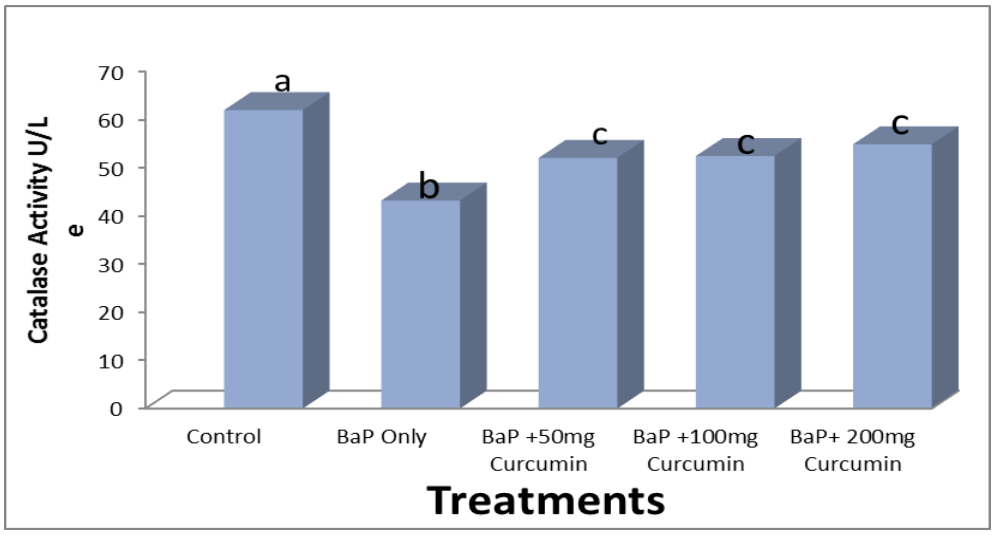

Figure 1: Effects Of Curcumin administration on the activities of Catalase in Benzo[a]pyrene treated albino rats.

Effects of curcumin administration on activities of superoxide dismutase in benzo[a]pyrene treated albino rats: Treatment of the rats with benzo[a]pyrene alone significantly reduced the activities of superoxide dismutase in them (Figure 2). Significant increases in the activities of this enzyme was observed following the administration of Curcumin at the different doses on the B[a]P-treated rats.

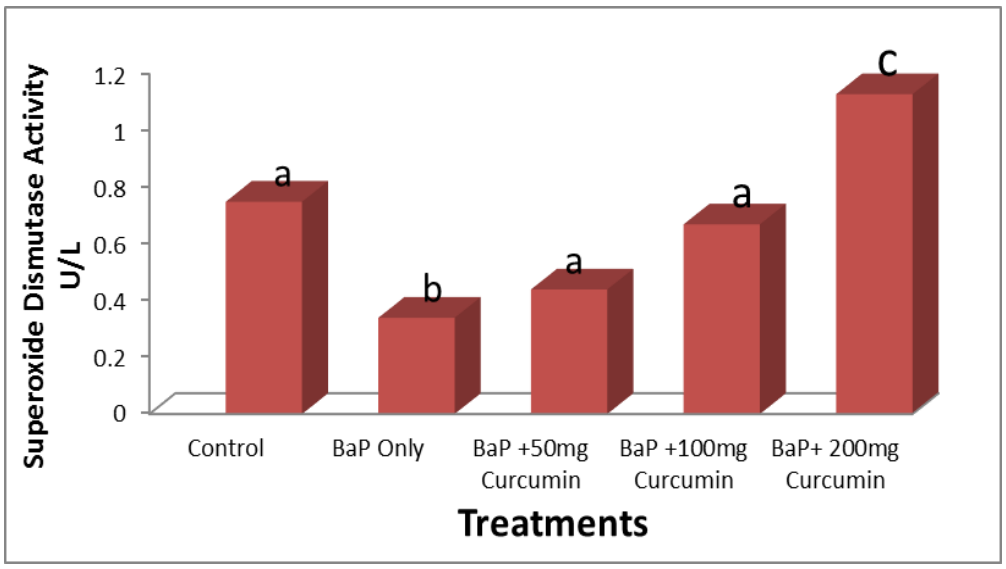

Figure 2: Effects of curcumin administration on activities of superoxide dismutase in benzo[a]pyrene treated albino rats

Effects of curcumin administration on activities of reduced glutathione (GSH) in benzo[a]pyrene treated albino rats: Rats administered benzo[a]pyrene alone had significantly ($P<0.05$) decreased specific activities of reduced glutathione (GSH) (Figure 3). Administration of Curcumin at different doses on these rats brought about significant increases in the activities of this enzyme.

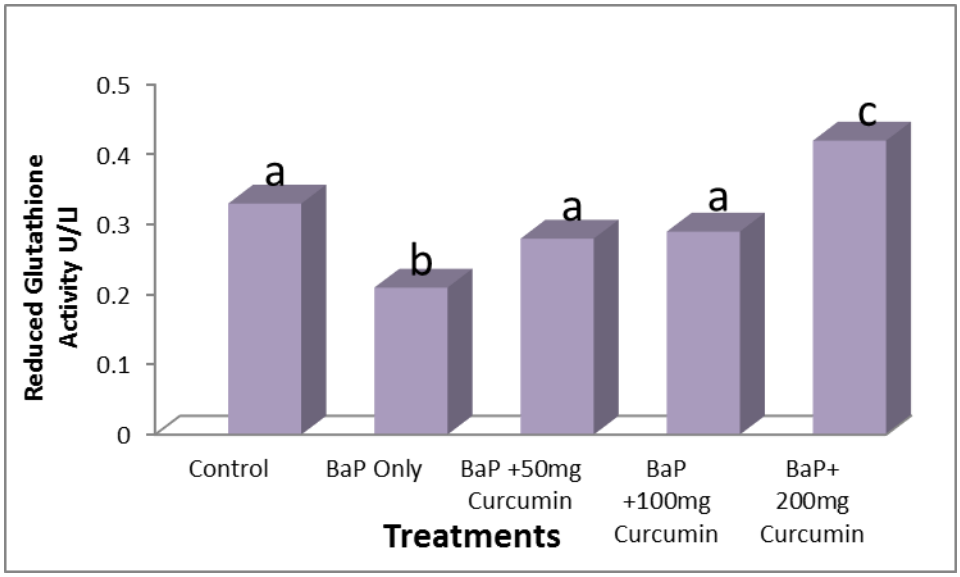

Figure 3: Effects of curcumin administration on activities of reduced glutathione (GSH) in benzo[a]pyrene treated albino rats

Effects of curcumin administration on activities of glutathione peroxidase (GPx)in benzo[a]pyrene treated albino rats: The activities of glutathione peroxidase(GPx) in the animals were significantly ($P<0.05$) reduced following the administration of 1 mg/kg body weight of B[a]P-alone on the rats (Figure 4). Significant increases in the activities of these enzyme was observed following the administration of Curcumin at the different doses on the B[a]P treated rats.

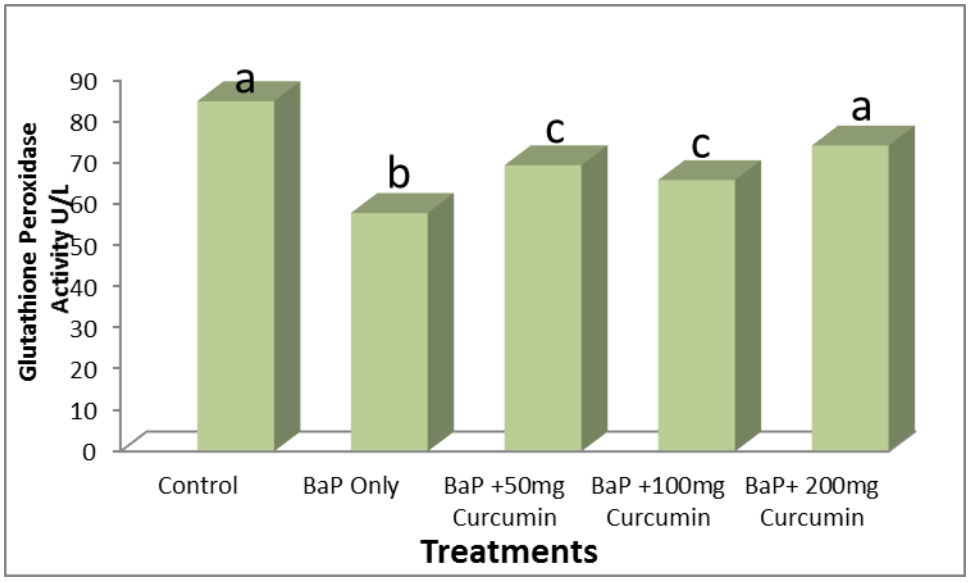

Figure 4: Effects of Curcumin administration on activities of glutathione peroxidase (GPx) in benzo[a]pyrene treated albino rats.

Effects of curcumin administration on concentrations of malondialdehyde in benzo[a]pyrene treated albino rats: Administration of benzo[a]pyrene alone significantly ($P<0.05$) increased the concentration of malondialdehyde, a lipid peroxidised product. (Figure 5). This trend was reversed when Curcumin was administered at various doses on B[a]P-treated rats. The rats co-treated with B[a]P and Curcumin at the different doses had malondialdehyde concentrations that compared favorably ($P>0.05$) with that of control.

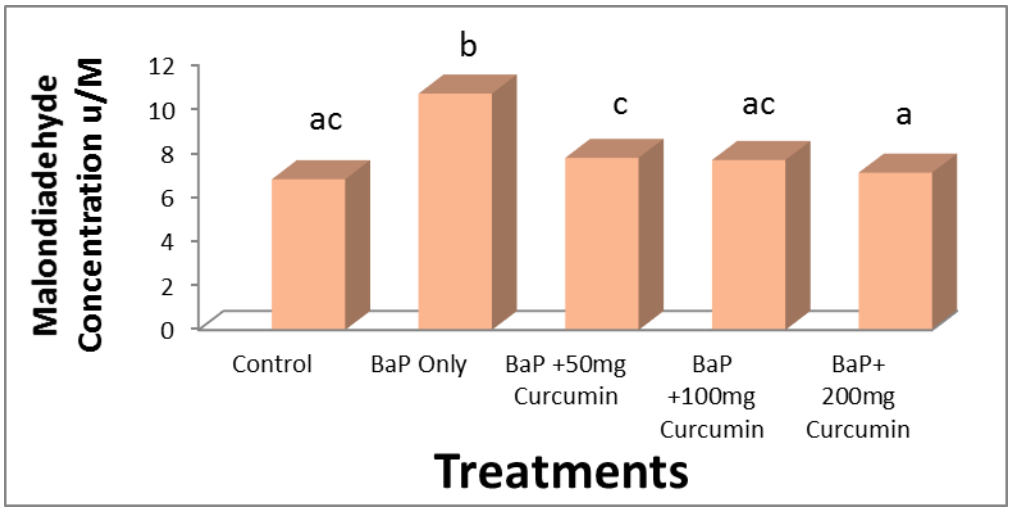

Figure 5: Effects of Curcumin administration on concentrations of malondialdehyde in benzo[a]pyrene treated albino rats

Discussion

Reports have shown that uncontrolled and excessive production of reactive oxygen species (ROS) can have damaging effects for cells even as these species are produced continuously in all living cells during the process of metabolism (Rezaei-Moghadam *et al*., 2012). B(a)P, a well known carcinogen formed during food-processing, and in tobacco smoke, waste products (Ali *et al*., 2017; Roh *et al*., 2012) generates enormous amounts of free radicals, which in turn reacts with lipids causing lipid peroxidation (Sikkim and Mulee,2000). Natural antioxidants from plant sources, tend to possess the ability of hindering the ROS production and thus limiting the associated intracellular oxidative stress (Feng *et al*., 2001).

In this study, B(a)P administration elicited significant increase in the lipid peroxidation as indicated by high levels of maloniadehyde (MDA) in serum (Figure 5). Benzo[a]pyrene, a cancer-causing agent, is metabolized in the liver tissues thus generating free radicals and oxidized metabolites by P450 enzyme system (Kim *et al*., 1998; Miroslav *et al*., 2016). These free radicals and non-oxidizing species are highly reactive and damaging, as they possess capacity to alter genetic material (Selvendiran *et al*., 2004); and are involved in triggering tissue lipid peroxidation. Therefore treatment with Curcumin brought significantly decreased levels of MDA in animals earlier treated with B(a)P. The ameliorating effects on the lipid peroxidation and ROS levels due to Curcumin treatment, may be attributed to its antioxidant and free radical scavenging properties based on its having both phenolic and diketone groups in its chemical structure; therefore, possessing free radical scavenging activities (Kakkar and Kaur, 2011). It may also have been via inhibition of reactive oxygen species generated by suppressing cytochrome P450 isozymes which are involved in the bioactivation of benzo[a]pyrene to toxic reactive metabolites such as B[a]P-7,8-diol-9,10-epoxide (BPDE).

The ability of a cell or tissue to maintain its integrity depends largely on the levels of the antioxidants present when compared with the level of the oxidants. The balance between these two determines the susceptibility of the cell or tissue to free radicals attack or oxidative stress. Results of our study further showed that the activities of the antioxidant enzymes [Superoxide dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx)] were significantly decreased in the benzo[a]pyrene-treated rats. However, supplementation with Curcumin significantly helped to the increase activities of these enzymes. This may be attributed to Curcumin's ability to enhance the activities of such antioxidant enzymes (Saad, 2013; AbdEl-Rahman and Al-Jamee, 2014).

Compared with the control, GSH concentrations in Wistar rats were significantly decreased after exposure to Benzo[a]pyrene. Liu *et al*. (2015) also reported a significant decrease in GSH levels following BaP treatment on mice. This might be due to the utilization of GSH in detoxifying the peroxides generated as a result of increased levels of lipid peroxidation. This depletion of GSH suggests a deleterious effect of benzo[a]pyrene on the antioxidant defence in the liver of the rats. It is also consistent with the occurrence of oxidative stress, thus emphasizing the role of GSH in molecular protective mechanisms that modulate cellular responses to toxic chemicals. Results of the present study further showed that administration of Curcumin helped to improve the GSH levels in rats. This is similar to findings of Otuechere *et al*. (2014), who indicated an increase of the GSH level in rats fed Curcumin. Reports have indicated that Curcumin increased the concentration of GSH as well as

boost the gene expression of SOD (Alia *et al*., 2006; Malik and Mukherjee, 2014; Jagetia and Rajanikant, 2015), based on Curcumin's activity as an antioxidant (Haryuna *et al.,* 2017). Curcumin also enhances the activities of other antioxidant enzymes such as superoxide dismutase, catalase, Glutathione Peroxidase (Saad, 2013; AbdEl-Rahman and Al-Jamee, 2014).

Conclusion

Results of the study showed that Curcumin could reverse the oxidative alterations induced by benzo(a)pyrene in Wistar rats through ROS regulation.

References

Abd El-Rahman SN, Al-Jamee SS: Protection of curcumin and curcumin nanoparticles against cisplatin induced nephrotoxicity in male rats. Sch Acad J Biosci, 2: 214-223. 2014.

Adeneye AA: Hypoglycemic and hypolipidemic effects of methanol seed extract of Citrus *paradisi Macfad (Rutaceae*) in alloxan-induced diabetic Wistar rats. Nig Q J Hosp Med, 18(4): 211–215. 2008.

Aladag I, Eyibilen A, Guven M, Atis O, Erkorkmaz U: Role of oxidative stress in hearing impairment in patients with type two diabetes mellitus. J Laryngol Otol, 123(9):957–963. 2009.

Ali N, Ismail IMI, Khoder M, Shamy M, Alghamdi M, Al Khalaf A, Costa M: Polycyclic aromatic hydrocarbons (PAHs) in the settled dust of automobile workshops, health and carcinogenic risk evaluation. Sci Total Environ, 601–602: 478–484. 2017.

Alia M, Mateos R, Ramos S, Lecumberri E, Bravo L, Goya L: Influence of quercetin and rutin on growth and antioxidant defense system of a human hepatoma cell line (HepG2). Eur J Nutr 45: 1928. 2006.

Aygün SF, Kabadayi F: Determination of benzo[a]pyrene in charcoal grilled meat samples by HPLC with fluorescence detection. Int J Food Sci Nutr, 56: 581-585. 2005.

Briede J, Godschalk RW, Emans MT: *In vitro* and *in vivo* studies on oxygen free radical and DNA adduct formation in rat lung and liver during benzo[a]pyrene metabolism [J], Free Radic Res 38(9): 995–1002. 2004.

Çelik S, Baysal B, Şen S: Resveratrol attenuates benzo(a)pyrene-induced dysfunctions, oxidative stress and apoptosis in pancreatic beta-cells. Adv Biosci Biotechnol, 10: 389-404. 2019.

Chiarugi P: Reactive oxygen species as mediators of cell adhesion. Ital J Biochem, 52: 31–35. 2003.

Costa C, Catania S, De Pasquale R, Stancelli R, Scibano GM, Melchini A: Exposure of human skin to Benzo[a]pyrene: role of CYP1A1 and aryl hydrocarbon receptor in oxidative stress generation. Toxicology, 271: 83–86. 2010.

Delgado-Saborit JM, Stark C, Harrison RM: Carcinogenic potential, levels and sources of polycyclic aromatic hydrocarbon mixtures in indoor and outdoor environments and their implications for air quality standards. Environ Int, 37: 383–392. 2010.

Dutta K, Ghosh D, Nazmi A, Kumawat KL, Basu A: A common carcinogen benzo[a]pyrene causes neuronal death in mouse via microglial activation. PLoS One, 5(4): e9984. 2010. doi: 10.1371/journal.pone.0009984. PMID: 20376308; PMCID: PMC2848611. 2010.

Ellman GL: Tissue sulphydryl groups; Arch Biochem Biophys, 82:70-77. 1971.

Feng Q, Kumagai T, Torii Y, Nakamura Y, Osawa T, Uchida K: Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress. Free Radic Res, 35: 779–788. 2001.

Gelboin HV: Benzo [alpha] pyrene metabolism, activation and carcinogenesis: Role and regulation of mixed-function oxidases and related enzymes. Physiol Rev, 60: 1107–1166. 1980.

Haryuna TS, Munir D, Maria, A, Bashiruddin J: The antioxidant effect of curcumin on cochlear fibroblasts in rat models of diabetes mellitus. Iran J Otorhinolaryngol. 29(93):197–202. 2017.

Jagetia GC, Rajanikant GK: Curcumin stimulates the antioxidant mechanisms in mouse skin exposed to fractionated gamma-irradiation. Antioxidants (Basel). 4(1):25–41. 2015.

Kakkar V, Kaur IP: Evaluating potential of curcumin loaded solid lipid nanoparticles in aluminium induced behavioural, biochemical and histopathological alterations in mice brain, Food Chem Toxicol, 49(11): 2906–2913. 2011.

Kim JH, Stansbury KH, Walker NJ, Trush MA, Strickland PT, Sutter TR. (1998). Metabolism of benzo[a]pyrene and benzo[a]pyrene-7,8-diol by human cytochrome P450 1B1. Carcinogenesis 19: 1847–1853.

Kono Y: Generation of superoxide radical during auto oxidation of hydroxylamine and an assay for superoxide dismutase. Arch Biochem Biophys. 186:189-195. 1978

Lee BM, Park KK: Beneficial and adverse effects of chemopreventive agents. Mutat Res. 523–524: 265-270. 2003.

Liu Y, Wu YM, Zhangi PY: Protective effects of curcumin and quercetin during benzo(a)pyrene induced lung carcinogenesis in mice. Eur Rev Med Pharmacol Sci 19:1736-1743. 2015.

Luck H: Catalase. In: Bergmeyer, H.U., Ed., Method of Enzymatic Analysis, Academic Press, New York and London, pp.885-894. 1965. http://dx.doi.org/10.1016/B978-0-12-395630-9.50158-4

Malik P, Mukherjee TK: Structure-function elucidation of antioxidative and prooxidative activities of the polyphenolic compound curcumin. Chin J Biol. Article ID 396708. pp. 1–8. 2014. | https://doi.org/10.1155/2014/396708

Miroslav S, Radek, I, Moserov M, Heinz HS, Eva F, Volker MA, Stiborov M: The Impact of individual cytochrome P450 enzymes on oxidative metabolism of benzo[a]pyrene in human livers. Environ Mol Mutag 57:229-235. 2016.

Otuechere CA, Abarikwu SO, Olateju V, Animashaun AL, Kale OE: (2014) protective Effect of Curcumin against the Liver Toxicity Caused by Propanil in Rats. Hindawi Publishing Corporation International Scholarly Research Notices Volume 2014, Article ID 853697. 2014.

Ranjit S, Sinha N, Kodidela S, Kumar S: Benzo(a)pyrene in cigarette smoke enhances HIV-1 replication through NF-κB activation via CYP mediated oxidative stress pathway. Sci Rep 8, 10394 2018. https://doi.org/10.1038/s41598-018-28500-z

Rezaei-Moghadam A, Mohajeri D, Rafiei B, Dizaji R, Azhdari A, Yeganehzad M, Shahidi M, Mazani M: Effect of turmeric and carrot seed extracts on serum liver biomarkers and hepatic lipid peroxidation, antioxidant enzymes and total antioxidant status in rats. Bioimpacts, 2(3), 151-157 2012.

Roh T, Kwak MY, Kwak EH, Kim DH, Han EY, Bae JY, Bang du Y, Lim DS, Ahn IY, Jang DE: Chemopreventive mechanisms of methionine on inhibition of benzo(a)pyrene-DNA adducts formation in human hepatocellular carcinoma HepG2 cells. Toxicol. Lett. 208: 232–238. 2012.

Saad TM: Effect of curcumin on some heavy metals induced renal and testicular injuries in male rats. Egypt J Hosp With. 53: 770-781. 2013.

Sak K: Cytotoxicity of dietary flavonoids on different human cancer types. Pharmacogn. Rev. 8:122–146. 2014.

Selvendiran K, Senthil kanthan P, Magesh V, Sakthisekaran D: Modulatory effect of piperine on mitochondrial antioxidant system in benzo[a]pyrene. Induced experimental lung carcinogenenis. Phytomedicine, 11. 85-89. 2004.

Sikkim H, Mulee B: Lipid peroxidation and antioxidant enzymes in the blood of rats treated with benzo(a)pyrene. Chem Biol Interac. 127: 139-150. 2000.

Tong GC, Cornwell WK, Means GE: Reactions of acrylamide with glutathione and serum albumin. Toxicol Lett. 147(2): 127-131. 2004.

Wills, ED: Mechanism of lipid peroxide formation in animal tissues. Biochem J. 99: 667-676. 1996.