Research in Pharmacy 1(4) : 22-27, 2011 ISSN : 2231-539X
www.researchinpharmacy.com

Regular Article

Benzo[a]pyrene induced liver and kidney cancer in swiss albino mice

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This study has been undertaken to find out the carcinogenic effect of benzo[a]pyrene induced oxidative damage in liver and kidney of swiss albino mice. Adult male swiss albino mice were exposed to benzo[a]pyrene (dissolved in corn oil) twice a week for a period of 60 days and 90 days (50mg /kg body weight). Level of lipid peroxidation and activities of enzymic antioxidants and non-enzymic antioxidants were studied. The results indicates that there is a marked elevation in lipid peroxidation of both target organs (livers and kidney), with a elevated level of GPx in kidney and deminished level of GPx in liver. The results also showed a decreased activities of SOD, CAT and reduced glutathione in liver and kidney. All these study concludes that benzo[a]pyrene acts as a carcinogenic agent in liver and kidney of swiss albino mice.

Keywords: Superoxide dismutase, catalase, glutathione peroxidase, reduced glutathione.

About 30% of all the cancer deaths are due to consumption of tobacco products and all the cancers of human are related to diet (Pyeifer *et al.,* 2002; Hecht *et al.,* 2005) but the complete avoidance of cancer- causing foods is difficult. Benzo[a]pyrene is considered a prototype polycyclic aromatic hydrocarbon (PAH), classic DNA damaging agent and carcinogen. During metabolism, benzo[a]pyrene are directly (or) indirectly metabolized into free radicals in liver (Sullivan, 1985). Free radicals are highly reactive oxygen-containing molecular species these includes superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). They are also generated as a byproducts of normal metabolism (Halliwell, 1991). Benzo[a]pyrene intoxication in various studies has demonstrated that it generates free radicals in many tissues which are responsible for the induction of many diseases including cancer in lung, liver and kidney (Gad and Hassan, 1999; Rodrigo and Rivera, 2001). Normally there is a critical balance between free radical generation and antioxidant defenses (Halliwell, 1994).

Here, liver and kidney toxicity was induced by administering benzo[a]pyrene into experimental adult male swiss albino mice and the level of lipid peroxidation, activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase and non-enzymic antioxidant like reduced glutathione are measured. In the present study, we report on the carcinogenic activity of benzo[a]pyrene on liver and kidney tissues of adult mice.

Materials and methods

Chemicals: Benzo[a]pyrene was obtained from sigma chemical company, S.T.Louis, Mo, USA. All other chemicals like used were of analytical grade.

Animals: Healthy adult male swiss albino mice (6-8 weeks old) were used thoroughout the study. The animals were maintained in a controlled environmental condition of temperature and humidity with an alternating 12h light/ dark cycles. And all the animals were fed with standard pellet diet (Gold mohor rat feed, MS. Hindustan lever Ltd., mumbai) and water ad libitum.

Dosage and treatment: Swiss albino mice were divided into three groups each containing four mice.

Group I- received only corn oil orally.

Group II- received benzo[a]pyrene in corn oil vechicle only - twice a week for 60 days. [50mg /kg body weight]. (Pandi, *et al*., 2011).

Group III- received benzo[a]pyrene in corn oil vehicle only – twice a week for 90 days [50mg /kg body weight].

After the respective treatment periods, on day 60 and 90, animals were fasted overnight and dissected under, ether anaesthesia by cervical decapitation. Their liver and kidney tissues were used for the biochemical analysis.

Preparation of supernatent: Immediately, liver and kidney tissue were removed, weighed and washed in ice-cold 1.15% Kcl and homogenised in a homogenizing buffer (50 mM Tris-Hcl, 1.15% KCl pH 7.4) using a teflon homogenizer. The homogenate was centrifuged at 9,000g for 20 minutes to give a 20% (w/v) tissue suspension. After centrifugation, the homogenate was used for various biochemical analysis.

Determination of enzymatic antioxidants: Lipid peroxidation was assayed by the method of (Ohkawa *et al.,* 1979). Superoxide dismutase (SOD, EC 1.15. 1.1) was assayed by the method of Marklund and Marklund *et al.,* (1974). The values of SOD activity are expressed as U/mg/protein. The activity of catalase (CAT, EC 1.11. 1.6) was determined by the method of Sinha et al. (1972). The values of CAT acticity are expressed as μmol of H_2O_2 utilized / min/mg protein. The activity of glutathione peroxidase (GPx, EC.1.11. 1.9) was determined by the method of Sharma et al. (2001) using hydrogen peroxide as substrate in the presence of reduced glutathione. The values of GPx are expressed as μmol of GSH utilized /min/ mg protein.

Determination of non-enzymatic antioxidants: Reduced glutathione (GSH) was assayed by the method of Moron et al., (1979). The concentration of GSH was expressed as by μg /mg protein.

Statistical analysis: Results are expressed as mean ± S.D (n=4). Comparisons between the means of the control and treated groups were made using one way analysis of variance (ANOVA) using the SPSS software package for windows.

Results

Table 1 describes the alternation in the level of lipid peroxidation on benzo[a]pyrene induced experimental animal groups and control. A profound increase was noticed in the level of LPO with a significant P<0.01 in group II and group III animals than those of control animals. On benzo[a]pyrene induction, there was a significant increase in both the tested organs (liver and kidney).

Table 1. Effect of LPO on liver and kidney of control and experimental mice

	Group I	Group II	Group III
Liver			
LPO	1.91 ±0.17	2.24±0.25* a	3.8±0.35 * b
Kidney			
LPO	0.97±0.08	1.31±0.09* a	1.64±0.10 *b

Values are expressed as mean ± S.D (n=4); a: as compared with control;
b: as compared with control; *: significance at the level of p<0.01.

Table 2 depicts the enzymatic antioxidant activities in the liver and kidney of control and experimental animals. The activities of SOD, CAT were significantly decreased (Ramakrishnan *et al.,* 2007 and Vinodhkumar *et al.,* 2006) but GPx activity was markedly increased in the kidney and decreased in the liver (Showkat Ahmad Ganie *et al.,* 2011*)* (P<0.05) of induced animals when compared with control animals.

Table 2: Effect of B[a] pyrene on the activity of enzymic antioxidant such as superoxide dismutase (SOD), Catalase (CAT) and Glutathioine peroxidase (GPx) in control and experimental groups of liver and kidney

	Group I	Group II	Group III
Liver			
SOD	4.17±0.38	3.65±0.42* a	3.07±0.34* b
CAT	47.26±5.0	29.52±2.27*a	22.81±2.24* b
GPX	6.41±0.72	5.20±0.43*a	5.0±0.53* b
Kidney			
SOD	2.16±0.20	1.94±0.19* a	1.64±0.11* b
CAT	65.97±6.5	54.60±6.2*a	34.15±3.5* b
GPX	2.48±0.35	2.97±0.34* a	3.15±0.031*b

Values are expressed as mean ± S.D(n=4); Unit of SOD : U/mg protein;
Unit of CAT : µmol of H_20_2 utilized/min/mg protein; Unit of GPX: µmol of GSH consumed/min/mg protein;
*-- P<0.05 significance; a—Group II compared with Group I; b—Group III compared with Group I

Table 3 represents the concentration of non-enzymic antioxidants in the liver and kidney of control and experimental animals. Benzo[a]pyrene administration markedly decreased the levels of reduced glutathione (P<0.05) in both the kidney and liver tissue when compared with control animals.

Table 3: The effect of B[a] p on the activity of non enzymic antioxidant Glutathione in control and experimental groups of liver and kidney

	Group I	Group II	Group III
Liver Glutathione	3.14±0.25	2.88±0.34* a	2.54±0.23 *b
Kidney Glutathione	4.32±0.42	3.9±0.39* a	2.9±0.34 *b

Values are expressed as mean ± S.D(n=4); Unit of GSH are expressed as µg/mg protein;
*-- P<0.05 significance; a—Group II compared with Group I; b—Group III compared with Group I

Discussion

Benzo[a]pyrene has been identified as a major risk factor for liver and kidney related cancer. Benzo[a]pyrene when administrated it gets distributed and deposited in organs such as the lungs, liver, kidney and heart. The reactive metabolite 7,8-diol-9,10- epoxide-benzo[a]pyrene has been formed from the metabolic conversion of benzo[a]pyrene by cytochrome P-450 (Gelboin, 1980). As this reactive metabolite reacts very rapidly with O_2 and forms more reactive free radicals (Selvendisan *et al.,* 2004). These free radicals causes oxidative damage in vital organs like liver and kidney. They have the capacity to initiate the peroxidation of membrane polyunsaturated fatty acids, necrosis of cell, reduced glutathione, damage to membrane and loss of antioxidant enzyme activity.

In this experimental study we investigated the change in the level of biomarkers. These markers are the important indices for the diagnosis of dysfunction in hepatic and kidney (Vijayakumar *et al.,* 1997). From this it is revelated that cells are damaged, cellular leakage and loss of functional integity of cell membrane in both tested organs.

Benzo[a]pyrene has been found to elevate lipid peroxidation in tissues. Lipid peroxidation is an important event to cell death and has been reported to cause serve impairment of membrane functions through increased membrane permeability and membrane damage, cytotoxicity and eventually cell death. The free radicals reacts with lipids and generates LPO which is involved in the formation of tumours. (Cigremis *et al*., 2004).

The two important enzymic antioxidants are SOD and CAT that reacts aganist the free radicals such as superoxide (O_2) and hydroxyl ions (OH^-). SOD is an enzyme containing copper (cu^{2+}) and zinc (zn^+) as cofactors that converts superoxide radical into hydrogen peroxide (Vamajuchi *et al.,* 1994, Anbarasi *et al.,* 2006) and molecular oxygen and thus protects the cells from oxidative damage caused by H_2O_2 and OH^-(Bolann and Ulvik 1991; Fridovich, 1986). In this study, SOD activity was significantly decreased in both liver and kidney of mice who exposed to benzo[a]pyrene.

CAT is a hemoprotein, localized in peroxisomes or microperoxisomes. This also catalyses the decomposition of H_2O_2 to H_2O and O_2 thus protecting the cells from oxidative damage caused by H_2O_2. In the present experiment we also observed a similar decrease in CAT activities of the liver and kidney tissues on benzo[a]pyrene exposure. Thus both SOD and CAT activities are decreased on exposure to benzo[a]pyrene. (Anbarasi *et al.,* 2006, Ramesh *et al.,* 2008; Ramesh *et al.,* 2007).

GPx is an enzyme containing four selenium has a cofactors that catalyses the breakdown of H_2O_2 and organic hydroperoxides into H_2O and O_2. Thus it plays a significant role in

protecting cells against the free radicals and carcinogenic chemicals by scavenging the free radicals. In this study, GPx activity was significantly decreased in the liver and elevated in the kidney of mice exposed to benzo[a]pyrene. (Ramesh *et al.,* 2010).

Glutathione a non enzymic antioxidant is a major low molecular weight non-protein thiol in living organisms. It plays a role in body's antioxidant defence against free radicals, peroxides and other toxic compounds (Sies, 1992). In our study the glutathione level was significantly lowered in the liver and kidney of experimental mice exposed to benzo[a]pyrene when compared to control group. This, glutathione depletion increases the sensitivity of cells and leads to tissue disorder and injury, thus causing tumour in liver and kidney (Limon Pacheco *et al.,* 2007).

In conclusion, the obtained results showed that benzo[a]pyrene induced oxidative damage on the liver and kidney by enhancing lipid peroxidation and diminishing the enzymic and non-enzymic antioxidant status. Thus, the results of our investigation suggest that liver and kidney are more prone to oxidative stress against benzo[a]pyrene induced toxicity.

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