

Original Article

## THE EFFECT OF BENZO(A)PYRENE ON GLUTATHIONE S-TRANSFERASE, GLUTATHIONE PEROXIDASE IN THE LIVER AND KIDNEY MOUSE *MUS MUSCULUS*

Parichehr Hanachi <sup>1</sup>, Shamaan NA <sup>2</sup>, Ramli J <sup>3</sup>, Arshad JH <sup>4</sup> & Syed MA <sup>5</sup>

### ABSTRACT:

The effect of benzo(a)pyrene (BaP) on the activities of glutathione S-transferase (GST) and glutathione peroxidase (GPx) and histology in the liver and kidney of the mouse, *Mus musculus* were investigated. Three treatment groups of mice (6 in each group) were injected with 200mg/kg BaP once a week. Groups 1,2 and 3 were sacrificed after two, four and eight weeks, respectively by cervical dislocation. Similarly three control groups of mice injected with corn-oil were sacrificed at week 2, week 4 and week 8. Sections of the liver and kidney of mice were stained with hematoxylin and eosin. The GST activity in the liver and kidney was significantly increased after two weeks compared to the control group ( $P<0.05$ ). The GPx activity in the liver significantly increased to a maximum at four weeks. After eight weeks, GST and GPx activities decreased in the treated mice liver compared to the control group. Grey-white hyperplastic nodules were visible in the liver after four weeks of treatment. The slides prepared from these tissues showed marked morphological changes where the hepatocytes were paler, more vacuolated and had lost their normal shape and arrangement. The kidney cells showed mild inflammation within the medulla and glomeruli after four weeks. After eight weeks severe inflammation was observed in medulla but there was no change in the other parts. In this study, it was found that differences in morphology and cellular changes in liver and kidney were time dependent. It is concluded that assay of GST activity provides a method to monitor antioxidant changes in mice liver and kidney after exposure to BaP. The result indicated that liver was main target for chemically induced toxicity.

**KEY WORDS:** Benzo(a)Pyrene, glutathione S-transferases, glutathione Peroxidase, histology of mouse liver and kidney, mouse *Mus musculus*.

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1. Dr. Parichehr Hanachi
2. Assoc. Prof. Dr. Nor Aripin Shamaan
3. Assoc. Prof. Dr. Johari bin Ramlia
4. Assoc. Prof. Dr. Juzu Hayati Arshad
5. Assoc. Prof. Dr. Mohd. Arif bin Syed
- 1-5. Department of Biomedical Sciences,  
Faculty of Medicine & Health Sciences,  
University Putra Malaysia,  
43400 UPM Serdang, Selangor, Malaysia.

### Correspondence:

Dr. Parichehr Hanachi  
Email: parichehr78@yahoo.com

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## INTRODUCTION

The most important problem that humanity is expected to face in this century will be environmental pollution. Polycyclic aromatic hydrocarbons (PAH) are abundant pollutants, and many PAH are carcinogenic, but only after metabolic activation. Polyaromatic hydrocarbons (PAH) have been known to be carcinogenic for more than 70 years. The most important PAH is benzo(a)pyrene (BaP). BaP is a widespread environmental contaminant and can be found in air, soil, water and a wide range of foods including fruits, vegetables, aquatic food and cooked food. The lethal dose

in mice from intraperitoneal administration was reported to be 500 mg/kg<sup>1</sup> and toxic dose low (TDLo) value of 300mg/kg for mice was found to produce cancer in different tissues.<sup>2</sup> BaP is among the most carcinogenic PAH<sup>3</sup> and it had been shown to influence enzyme activities and histology of tissues during carcinogenesis<sup>4</sup>. Histopathology has for a long time been used as a potential marker for environmental stress<sup>5</sup>. The pathological and inflammatory changes in liver and kidney are different among the treated animals with different xenobiotics<sup>6</sup>. Moreover, studies have shown that there are several apparent similarities in mouse and human tissues. Therefore, a mouse model can be used to understand glutathione S-transferase (GST) mediated detoxification in humans.

This study was initiated to investigate the effect of BaP on liver and kidney GST and glutathione peroxidase (GPx) in control mice and those treated with 200 mg/kg BaP and compare the morphological and histological changes with preneoplastic marker enzymes activity especially cytosol GST and GPx in the liver and kidney. The study was important for the assessment of the environmental hazards of BaP.

## MATERIAL AND METHODS

**Chemicals, Glutathione:** 1-chloro-2,4-dinitrobenzene and benzo(a)pyrene and all other reagents used were of highest grade commercially available (Sigma Chemical Co., St. Louis, Mo, USA).

**Treatment of animals:** The mice *Mus musculus* ICR strain were purchased from the Institute of Medical Research, Kuala Lumpur. Animal feed was obtained from the Coin Ltd, Kuala Lumpur. The 36 mice initially weighing ~ 25-32 g, 8-10 weeks old, were housed in wire cages in a ventilated room at 27 ° C and were given a basal diet. The mice were divided into six groups of six mice each. Three treatment groups were injected interaperitoneally (i.p) with 200 mg/kg BaP dissolved in corn oil once a week

while control groups was injected i.p with corn oil and then animals were sacrificed at two, four and eight weeks by cervical dislocation.

**Preparation of cytosol:** The cytosolic preparation was carried out essentially following the method of Speier and Wattenberg<sup>7</sup>. All of the following procedures were carried out at 4°C. The mouse liver and kidneys were weighed individually and washed with cold 1.15%KCl. The liver and kidney were homogenised in 1.15% KCl (ratio of KCl to tissue, 3:1 v/w) and centrifuged at 10,000g for 20 minutes. The resulting homogenate was filtered through glass wool and further centrifuged at 100,000g for 1 hr. The cytosolic fraction was used for the assays of GST and GPx activities and protein determination.

**Enzyme assays:** Glutathione S-transferase (GST) was assayed essentially by the method of Habig et al,<sup>8</sup>. One unit of GST activity is expressed as amount of enzyme required to conjugate 1 imol of the substrate with GSH per minute at 29° C.

Glutathione peroxidase (GPx) was assayed according to method of Lawrence and Burk<sup>9</sup>. The assay was based on the coupled reaction with glutathione reductase. The unit definition was the amount of enzyme which caused the oxidation of one micromole of GSH per minute at 25° C and pH 7. Protein concentration in cytosolic fractions of liver and kidney were determined according to the method of Bradford<sup>10</sup>.

**Histological studies:** Sections of liver, and kidney of mice, were fixed in 10% neutral buffered formalin, dehydrated and then embedded in paraffin wax. The samples were then put into tissue basket for histological blocking by using Histokinette 2000. Multiple sections from each block (5mm thick) were prepared and all sections were stained with hematoxylin and eosin. Slides were randomly coded to ensure unbiased interpretation and were examined using light microscope at 40x, 100x, 120x and 400x magnification.

**Statistical analysis:** The data obtained from experiment were analyzed by inferential statistic in terms of t-test and Analysis of Variance (ANOVA) in which post-hoc comparisons were made using the Benferonni's test. A  $p < 0.05$  was considered to be significant.

## RESULTS

The GST activity in liver was significantly increased after two weeks and similar trends were also observed in GST activity in kidney compared to the control group ( $p < 0.05$ ). However, the GST activity in liver was significantly decreased after eight weeks compared to the control group. The GPx activity in liver using  $H_2O_2$  as substrate was significantly increased to a maximum at four weeks (Table-I). GPx activity in kidney did not change significantly after two, four and eight weeks (Table-I). The morphology of the liver in the treated group was different after four weeks compared to the control group and showed generalized nodule formation. The normal hepatocytes were well arranged and uniformly stained (Fig 1). The liver after four weeks of treatment showed marked morphological changes. The hepatocytes were paler, more vacuolated and had lost their normal shape and arrangement (Fig 2). Liver sections of the mice for a period of 8

weeks showed the hepatocyte even more enlarged, paler, more vacuolated and had lost their normal shape and arrangement (Fig 3). A section of the control mice kidney showed that the cells were well arranged and uniformly stained (Fig 4), however, mild inflammation within the medulla and glomeruli was noticed after four weeks of treatment (Fig 5). Kidney sections of the treated mice also showed that the lymphocytes were abundant and there was more severe inflammation in medulla after eight weeks of treatment (Fig 6). No changes were observed in the other parts of the kidney.

## DISCUSSION

Antioxidant enzyme activities in the liver, kidney, lung and forestomach previously have been induced by intraperitoneal administration of BaP<sup>11,12</sup>. Liver plays a central role in toxicological response and pollutant-induced pathological changes. These can be observed at the level of liver histology<sup>5</sup>. In particular, the over-expression of GST in tumours appears to be a factor in the development of acquired resistance towards anticancer drugs and hence GST is a therapeutic target for rational drug design<sup>13</sup>. GST conjugation has been shown to be an important protection mechanism against DNA

Table-I: The specific activity of GST and GPx at 200 mg/kg BaP over time in the liver and kidney of normal and treated mouse

Enzyme	Organ *	2 weeks		4 Weeks		8 Weeks	
		Control	Treated	Control	Treated	Control	Treated
GST							
$\mu\text{mol}/\text{min}/\text{mg}$ protein							
	Li	3.8±0.33	4.1*±0.46	3.9±0.6	4.2±1.5	4.6±1.0	2.72*±0.78
	Ki	0.46±0.09	0.69*±0.1	0.67±0.07	0.6±0.1	0.52±0.12	0.67±0.12
GPx ( $H_2O_2$ )							
$\mu\text{mol}/\text{min}/\text{mg}$ protein							
	Li	0.58±0.1	0.62±0.11	0.56±0.13	0.73*±0.08	0.54±0.2	0.49±0.19
	Ki	0.72±0.08	0.76±0.06	0.98±0.21	0.81±0.24	0.53±0.18	0.42±0.18

Organ+ Li- Liver, Ki- Kidney

Values shown are mean ± SD (n=5-7) \*P+ <0.05 compared to control.

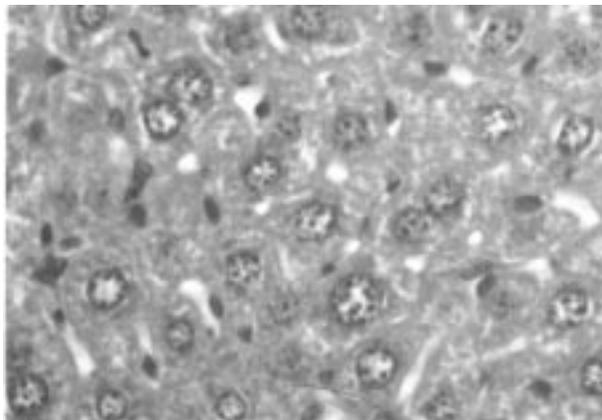


Fig 1: Liver section from control mice (Received corn oil) showing normal hepatocyte ( ➡ ). Magnification is 400 X.

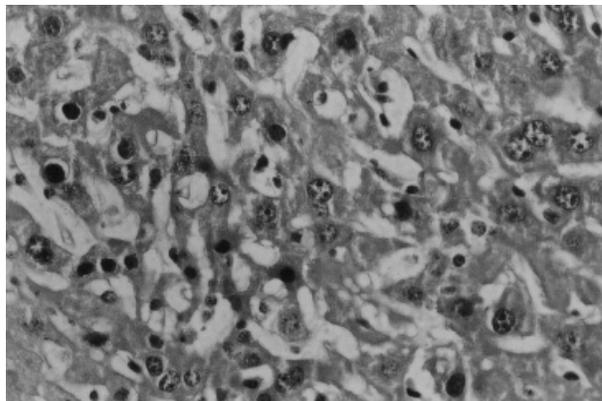


Fig 2: A photomicrograph of liver receiving doses 200 mg/kg the BaP every week over a period of 4 weeks. This photomicrograph shows hepatocytes with degeneration pyknotic nuclei ( ➡ ) Magnification is 400 X.

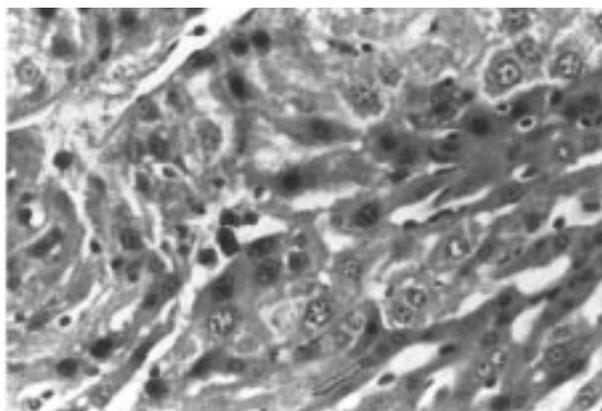


Fig 3: A photomicrograph of liver received a dose of 200 mg/kg BaP every week over period of 8 weeks. The hepatocytes shows degeneration and necrosis ( ➡ ). Magnification 400X.

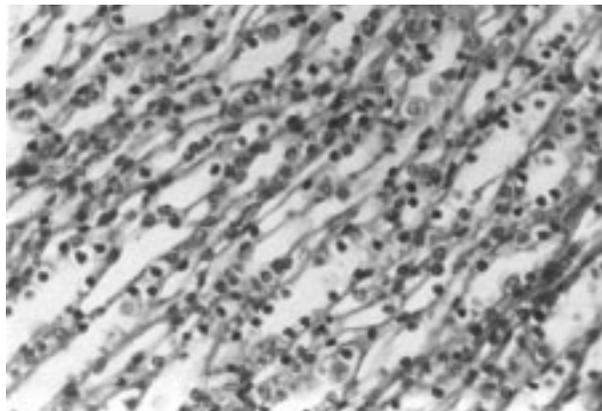


Fig 4: Kidney section from control mice (Received corn oil) showing normal kidney histology. Magnification 400 X.

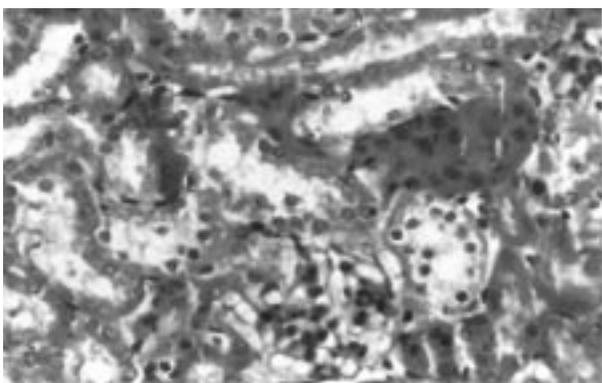


Fig 5: A photomicrograph of the kidney of mice which received 200 mg/kg BaP every weeks over a period of 8 weeks. Kidney section shows more severe inflammation and epithelialization ( ➡ ). Magnification 400X.

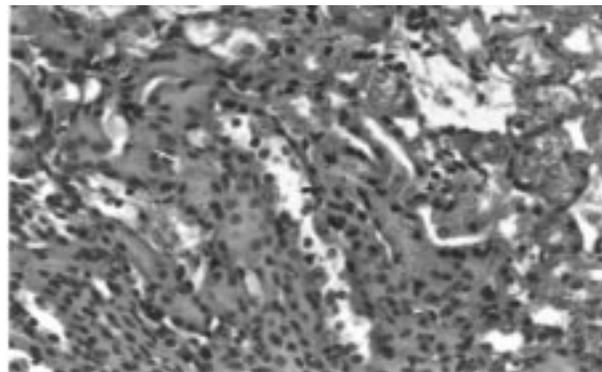


Fig 6: A photomicrograph of kidney of mice which received a dose of 200 mg/kg BaP every week over a period of 4 weeks. Kidney section showing mild inflammation (neutrophils and lymphocytes) within the medulla with necrotic cell ( ➡ ). Magnification is 400 X.

binding to the bay region of BaP. The hepatic GST appears to play an important role in reducing the BaP-induced DNA damage in target and non-target tissues<sup>14</sup>.

Feng et al<sup>15</sup> described the effect of different BaP concentrations on antioxidant enzymes. The result showed that the activities of antioxidant enzymes did not change too much with BaP exposure in lower concentration. The result obtained from the previous study in our laboratory indicated that a dose of 200 mg/kg BaP per week up to 8 weeks is an optimum dose to evaluate the GST and GPx activities in mice. The activity of GST in liver and kidney was significantly increased after 2 weeks compared to the control group. This may be due to the interaction of metabolites with cellular DNA. Thus, the treatment given to the mice was effective in inducing GST activities almost immediately. This had been reported by Lotlika<sup>16</sup> and Ketterer<sup>17</sup>. The levels of GST in liver and kidney were not increased when the mice were treated with BaP for 4 and 8 weeks. It might appear that the period of treatment in this case is too long a period for continued induction. The half time for turnover of glutathione in the liver is about 4 h, so that 4 and 8 weeks is a relatively long time. It is of interest to note that in treated mice, GST activity decreased dramatically in the liver after 8 weeks, suggesting that such induction may render both tissues more susceptible to carcinogenic effect of BaP. Therefore, the damaged liver cells are no longer capable of synthesizing GST protein. This might be of interest in view of the importance of the liver in the vital process of detoxification.

In this study it has been demonstrated that the activity of GPx markedly increased in liver after 4 weeks. It has been pointed out that GPx has a very important role in removing H<sub>2</sub>O<sub>2</sub> which is produced in liver of mice administered xenobiotics including carcinogens and may cause cell membrane damage. These results suggest that the increased activities of GPx towards H<sub>2</sub>O<sub>2</sub> and GST protect the cells in preneoplastic nodules from damage by H<sub>2</sub>O<sub>2</sub> and lipid peroxidase.<sup>18</sup>

## CONCLUSION

These results show that the increased activities of antioxidant enzymes, such as glutathione S-transferase and glutathione peroxidase play a major role in repairing the damage caused to cytoplasmic nuclear and endogenous membranes in liver. The liver is main target for chemically induced toxicity and several factors contribute to its being particularly susceptible. Firstly, it is the organ with the highest complement of cytochrome P450 in terms of quantity as well as number of isoenzymes. Secondly the liver is the first site for the metabolism of xenobiotics absorbed from GI tract. In this study, it was found that the differences in the morphology and cellular changes in liver and kidneys after exposure to BaP were time dependent.

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