UDC 576.882.821.234.097.29

ORIGINAL ARTICLES

Aflatoxin B_1-induced changes of glutathione--S-transferase activity in the plasma and liver of the rat

Nataša J. Strelić*, Zorica S. Saičić†, Zvonko M. Magić*, Mihajlo B. Spasić†, Nataša V. Trutić‡, Koviljka V. Krtolica§

Military Medical Academy, *Institute for Medical Research, Belgrade, Institute for Biological Research „Siniša Stanković", †Department of Physiology, Belgrade, University of Niš, Faculty of Medicine, ‡Institute of Medical Chemistry, Niš, §Institute of Nuclear Sciences - Vinča, Belgrade, Serbia & Montenegro

Background. *The influence of low doses of aflatoxin B_1 (AFB_1) and partial hepatectomy (PH) on glutathione-S-transferase (GST) activity was studied in the plasma and liver of the rat.* ***Methods.*** *The animals were divided into four groups. The first (I) and the second (II) group were treated with AFB_1 freshly dissolved in dimethyl sulphoxide (DMSO), and administered as a single intraperitoneal dose of 50 μg/rat 24 hrs after the rats had undergone either sham operation or, 40% PH, respectively. The third group (III) of animals was treated with a total dose of 1 mg AFB_1 – 5 days per week during a period of 8 weeks. The non-treated animals were used as controls (C).* ***Results.*** *We observed a significant increase of GST activity in the plasma of all experimental groups compared to the controls (C), ($p<0.02$ to $p<0.005$). In the liver, the GST activity of all experimental groups was also significantly increased, compared to the controls (from $p<0.02$ to $p<0.005$).* ***Conclusion.*** *The administration of both single and multiple doses of AFB_1 led to long term increase of GST activity in the rat plasma and liver, and partial hepatectomy had no significant effect on this phenomenon.*

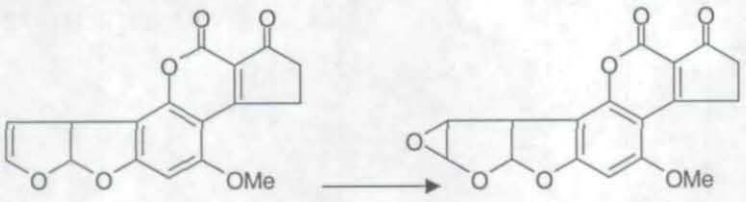
Key words: **aflatoxins; carcinogens; glutathione transferase; liver; plasma; rats.**

Introduction

Aflatoxins are widely distributed in the nature and the most important of these closely related mycotoxins is aflatoxin B_1 (AFB_1), which is a metabolite of certain *Aspergillus* species (*A. flavus* and *A. parasiticus*). These are widespread fungi, which can contaminate animal and human food (1). It is known that AFB_1 is hepatotoxic and hepatocarcinogenic in many animal species, including rats (2, 3), and also humans (4). The liver is an important target of the toxicity of drugs, xenobiotics and oxidative stress (5). Many chemical compounds are modified by liver extracts to yield active compounds (among these are epoxides), and their actions are usually directed to macromolecules such as DNA (6, 7). In order to exert its biological effects (acute toxicity, teratogenecity, mutagenicity and carcinogenicity), AFB_1 must be converted to its reactive epoxide (Figure 1) by cytochrome P450 enzyme system of liver and several other tissues of different animals (8, 9). The epoxide is highly reactive, and can form derivatives with several cellular macromolecules, including DNA, RNA, and proteins. It is believed that covalent interaction of epoxide with DNA is responsible for the initiation of carcinogenesis followed by the subsequent step of promotion and progression, and that it leads to cancer formation (7).

Hepatic antioxidants represent the major defence against toxic liver injury, and they act as anti-apoptotic agents (10).

With respect to the capacity for AFB, oxidation, striking differences exist among microsomes prepared from dif-



AFLATOXIN B_1 **AFLATOXIN B_1 - 8,9 EPOXIDE**

Fig. 1 – Conversion of aflatoxin B_1 to aflatoxin B_1 - 8,9 epoxide in the liver

ferent species. Human liver microsomes are approximately one-fourth as efficient in activating AFB_1 as rat microsomes. For many years it has been perceived that the mouse and rat, though closely related, respond in a completely different way to hepatocarcinogenic effects of aflatoxin B_1 (11). Mouse microsomes have higher specific activity for AFB_1-8,9-epoxide (AFBO) production than rat microsomes, but are resistant to the hepatocarcinogenic effects of AFB_1 because of the efficient conjugation of AFBO with glutathione (GSH) (12, 13). Glutathione-S-transferase (GST) is involved in detoxification of epoxides through conjugation between GSH and AFB_1 (14). GST is found in cytosol of cells in vertebrates, plants, insects, nematodes, yeasts, and aerobic bacteria. In mammalian organisms GST is present in all tissues, but the highest activity of this enzyme was found in the liver, representing up to 10% of total protein amount.

GST activity might be a key factor in determining individual or species susceptibility to AFB_1, and the major route of detoxification of AFB_1 via conjugation of AFBO with GSH is in rodents, noticeably.

Satoh *et al.* showed that hepatectomy enhanced tumor development in rats given chemical carcinogenes (15).

The aim of this study was to investigate the influence of low doses of AFB_1 and partial hepatectomy (PH) on GST activity in the plasma and liver of the rats.

Methods

Male rats of the inbred Albino Oxford (AO) strain (4–6 weeks old) were used in the experiment. Animals were kept in wire-bottomed cages under the standardized conditions of humidity, light and temparature at the Institute for Medical Research of the Military Medical Academy. Food and water were given *ad libitum*.

The animals were divided into four groups. The first (I) and the second (II) group were treated with AFB_1 freshly dissolved in dimethyl sulphoxide (DMSO), administered as a single intraperitoneal dose of 50 μg/rat (24 hrs after the rats underwent a sham operation, or 40% PH, respectively). The third group (III) of rats was treated with a total dose of 1 mg AFB_1 - 5 days per week during 8 weeks. Non treated animals were used as the controls (C).

Partial hepatectomy was performed according to the technique of Higgins and Anderson (16). The animals were sacrificed 13–15 months after the administration of AFB_1. The livers were excissed within 3 minutes and prepared for further analysis. All chemicals were the products of Sigma (St. Louis, MO, USA). Fresh blood was immediately collected using heparin (1000 U/ml) as anticoagulant. Aliquots of blood were taken immediately after exsanguinations and centrifuged for the separation of plasma. For the determination of GST activity in the plasma and liver 1-chloro-2,4-dinitrobenzene (CDNB) was used as a substrate (17). Protein content was determined by the method of Lowry *et al*, using bovine serum albumin as a referent value (18).

The obtained data were statistically analyzed and expressed as mean values ± SE, and differences between the experimental and the control group were estimated by Student's *t*-test (19). The value of $p<0.05$ was considered statistically significant.

Results

In our experiments we observed a significant increase of GST activity in the plasma of all three experimental groups compared to controls, as presented in Figure 2.

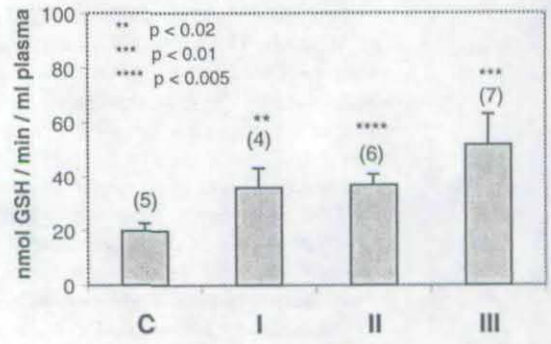


Fig. 2 – Changes of glutathione-S-transferase (GST) activity in the plasma of rats sacrificed 13–15 months after AFB_1 treatment.

AFB_1 was administered intraperitoneally to rats in the single dose of 50 μg/rat 24 hrs after the rats underwent a sham operation (I) or 40% partial hepatectomy (II) and in multiple doses 5 days/week (total doses of 1 mg AFB_1 in 8 weeks) (III). Nontreated animals represented controls(C). The number of animals is given in parentheses.

The sham operated rats 24 hrs before the treatment with AFB_1 showed lower than 2-fold increase in GST activity, compared to the control value ($p<0.02$). The most significant increase (near 2-fold, $p<0.005$) was in the group of rats II which underwent hepatectomy 24 hrs before the single treatment with AFB_1.The highest increase in GST activity in plasma was detected in the group of rats III, which were treated for two months with 1 mg of AFB_1 as a total dose (2.5-fold, $p<0.01$).

Changes of GST activity in the liver of the rats treated by AFB_1 are presented in Figure 3A and 3B. A significant

increase ($p<0.01$) of specific activity of GST from group II was found in comparison to the control animals (Figure 3A). When the activity of GST was expressed in gram of the wet mass of tissue (Figure 3B) the values were more significant in all three experimental groups of animals in respect to the control rats ($p<0.01$ and $p<0.005$).

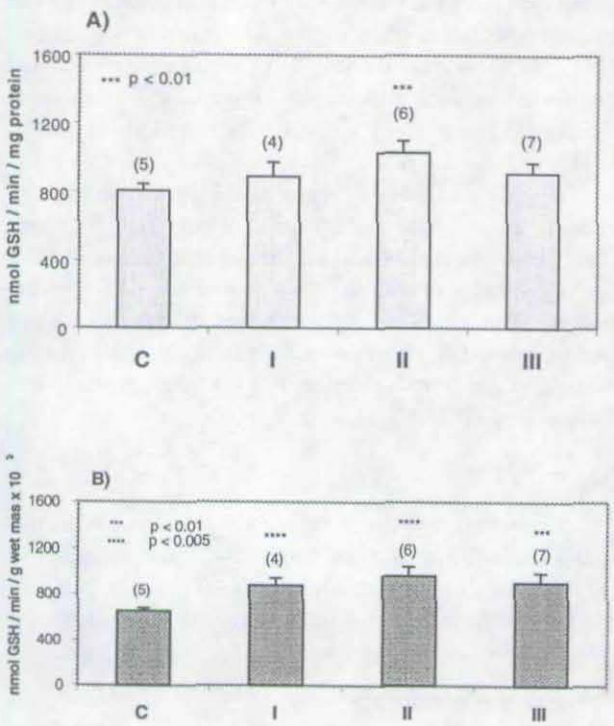


Fig. 3 – Changes of glutathione-S-transferase (GST) activity in the liver of rats after AFB_1 treatment

AFB_1 was administered intraperitoneally to the rats in the single dose of 50 μg/rat 24 hrs after the rats underwent a sham operation (I), or 40% partial hepatectomy (II), and in multiple doses 5 days/week (total doses of 1 mg AFB_1 in 8 weeks) (III). Nontreated animals represented controls (C). The number of animals is given in parentheses.

Discussion

Different studies in rats have demonstrated the potent toxic and carcinogenic effects of aflatoxins (2). Despite the differences, (different sources of aflatoxin - food contaminated with AFB_1 or purified AFB_1, different routes of administration, different periods of administration and observation, different basic diets and different strains of rats), all of these studies found that aflatoxin was a very potent hepatic carcinogen in rats fed for the period of 20 weeks, or longer. A few studies found that even single, relatively high doses were capable of producing hepatocellular carcinoma (12). The selected doses of AFB_1 that were used in our study were potentially cancerogenic (20, 21). Sublethal doses of aflatoxins led to chronic toxicity that caused cancerogenic changes. Lethal dose of AFB_1 varied and depended on strain difference, animal's age and other factors (22). Many of the earlier studies showed that multiple doses of AFB_1 led to the development of liver tumors in rats between the 1st and the 2nd year after poisoning (23). Other studies showed that PH combined with AFB_1 enhanced tumor development (20).

An important detoxification pathway in animals – the conjugation of AFB_1 to GSH (mediated by GST) and its subsequent excretion is related to AFB_1 toxicity resistence (24). Differences in AFB_1 susceptibility of various species depended on their hepatic cytosol capability to inactivate AFBO by conjugation with GSH (25, 26). Mice occured highly resistant to the hepatocarcinogenic effects of aflatoxin (27). Although mouse microsomes produced relatively more AFBO than rat microsomes under similar incubation conditions, mice were more resistant to the hepatocarcinogenic effects of AFB_1 because of the efficient conjugation of AFBO with GSH (28). Liver cytosolic fractions from the mouse had 50- to 100- fold greater AFBO conjugating activity than those from the rat (12).

Individuals with high activity of oxidative enzymes and/or low activity of detoxifying enzymes might be at the increased risk for certain types of cancers. For AFB_1 cancer risk estimation, the rates of cytochrome P450 (CYP)-mediated AFB_1 activation, as well as GST-catalyzed AFBO -detoxification must be concidered (29).

Slone *et al.* (28) analyzed hepatic cytosolic fractions prepared from 14 human donors for GST activity. Human liver cytosolic GST exhibited low activity towards AFBO (0.17-1.46 μmol/min/mg). Hepatic GST-AFBO activities of the rat, hamster and mouse were 70-, 465-, and 3545-fold greater, respectively, than the ones observed in human liver using microsomally-generated AFBO.

In our experiment, total hepatic GST activities towards AFBO measured one year after AFB_1 poisoning, were increased 10–40%, compared to the controls.

Earlier studies (15) with purified GST from the rat liver indicated different activity of certain class of this enzyme towards AFBO. In the direct contrast to a large number of other drug-metabolizing enzymes, GST-P (subunit of neutral GST purified from placenta) was found not to be inducible in the livers of rats by short-term administration of drugs or carcinogens such as phenobarbital, a butylated hydroxyanisole, *N*-2-fluorenylacetamide, 3-methylcholanthrene, 3-methyl-4-dimethylaminoazobenzene. However, two chemical carcinogens diethylnitrosamine, and *N*-2-fluorenylacetamide, plus partial hepatectomy induced preneoplastic foci and hyperplastic nodules in rat liver and elevated the amounts of GST-P 30–50 times, compared to normal liver (15). On the basis of their results, Satoh *et al.* concluded that GST-P might be expected to have a crucial role in relation to the resistance mechanism or, more directly, to the growth of preneoplastic cells.

Liu *et al.* (30) found persistent expression of GST-P in all the livers of AFB_1 -treated rats during three time intervals (1, 3, and 12 months) after AFB_1 poisoning. Since there were no liver tumors induced within one year after AFB_1

treatment, these data suggested that all AFB_1 -treated animals were at the preneoplastic stage of hepatocarcinogenesis. The GST-P positive foci thought represented the populations of the „initiated" cells with altered gene expression (15). AFB_1 induction resulted in GST-P overexpression, but not in the expression of *p53* tumor-suppressor gene that played an important role in hepatocarcinogenesis (30). These results suggested that the *p53* gene mutation might not have occured at this early stage of AFB_1 - induced hepatocarcinogenesis.

In our experiments neoplastic nodules and hepatocellular carcinoma were not detected in the group of rats II which underwent 40% PH 24 hrs before the single treatment with AFB_1 (results not presented). It occurred, that the strain difference and the timing of AFB_1 administration in relation to PH might have had a critical role in tumor development (31). The experiments in which rats underwent two thirds PH, and 24 hrs later received 0.25 mg/kg body weight of AFB_1, showed a significantly higher incidence of hepatocellular carcinoma, compared to non-hepatectomized rats sacrificed between the 55th and the 65th week (20). Mortality during AFB_1 administration was much lower when the rats underwent a one third hepatectomy (40%), than in the experiment in which more extensive hepatectomy was performed (70%) (31). In our experiments the mortality from hepatectomy was 70 % in AFB_1- treated rats.

In almost all the experimental systems, multiple dosing AFB_1 was required for carcinogenicity (13). A single administration of AFB_1 to the rats resulted in maximum liver AFB_1-DNA adduct levels 2 hrs after poisoning. The rapid removal of these adducts (88% after 24 hrs) might be related to the requirement for multiple exposures to AFB_1 for the induction of tumors in the Fischer rats (21).

The effect of chronic administration of AFB_1 on GST (hepatic phase II metabolizing enzyme) was measured one year after poisoning in the group III of animals treated with total dose of 1 mg AFB_1- 5 days/week in the period of 8 weeks. GST activity was significantly increased after multiple doses of AFB_1.

Guerre *et* al. (32) reported a significant decrease in cytosolic GST in a rabbits sacrificed 24 hrs after the last oral administration (0.10 mg/kg AFB_1 for 5 days). In rats GST activity measured with CDNB as a substrate occured unaffected by such a treatment, suggesting differences in the rat GST sensitivity to AFB_1 compared to the rabbit (33).

A variety of dietary factors have been shown to influence the carcinogenety of aflatoxin. Wang *et al.* (34) have found that crocetin (a natural carotenoid) enhanced GST activity in rat liver and protected against the AFB_1 hepatotoxicity. The incidence of liver lesions in male Wistar rats treated with AFB_1 (total dose of 1.125 mg/rat) and crocetin was significantly reduced (for 40%) in respect to the animals treated with AFB_1 alone.

Conclusion

Consistent with the obtained data we concluded that the administration of both single and multiple doses of AFB_1 led to a long time increase of GST activity in the rat plasma and liver, and that partial hepatectomy had no significant effect on this phenomenon.

Acknowledgements

This work was supported by the Military Medical Academy, Institute for Medical Research, Belgrade, and by the Ministry for Science and Technology of Serbia, grant Nos. 1669 and 2019.

REFERENCES

1. *Wild CP, Hall AJ.* Primary prevention of hepatocellular carcinoma in developing countries. Mutat Res 2000; 462(2–3): 381–93.
2. *Enemoto M.* Carcinogenicity of Mycotoxins. In: *Uraguchi K, Yamazaki M,* editors. Toxycology, biochemistry, and pathology of mycotoxins. Tokyo: Tokyo Press; 1984. p. 239–62.
3. *Kumagai S, Sugita-Konishi Y, Hara-Kudo Y, ItoY, Noguchi Y, Yamamoto Y,* et al. The fate and acute toxicity of aflatoxin B 1 in the mastomys and rat. Toxicon 1998; 36(1): 179–88.
4. *Hussein HS, Brasel JM.* Toxicity, metabolism, and impact of mycotoxins on humans and animals. Toxicology 2001; 167(2): 101–34.
5. *Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ.* Mechanisms of Hepatotoxicity. Toxicol Sci 2002; 65(2): 166–76.
6. *Modali R, Yang SS.* Specificity of aflatoxin B 1 binding on human proto-oncogene nucleotide sequence. Prog Clin Biol Res 1986; 207:147–58.
7. *Shen HM, Ong CN, Lee BL, Shi CY.* Aflatoxin B 1 induced 8-hydroxydeoxyguanosine formation in rat hepatic DNA. Carcinogenesis 1995; 16(2): 419–22.
8. *Sotomayor RE, Sahu S, Washington M, Hinton DM, Chou M.* Temporal patterns of DNA adduct formation and glutathione S-transferase activity in the testes of rats fed aflatoxin B 1: a comparison with patterns in the liver. Environ Mol Mutagen 1999; 33(4): 293–302.
9. *Liu L, Massey TE.* Bioactivation of aflatoxin B 1 by lipoxygenases, prostaglandin H synthase and cytochrome P450 monooxygenase in guinea-pig tissues. Carcinogenesis 1992; 13(4): 533–9.
10. *Meki AR, Abdel-Ghaffar SK, El-Gibaly I.* Aflatoxin B 1 induces apoptosis in rat liver: protective effect of melatonin. Neuroendocrinol Lett 2001; 22(6): 417–26.

11. *Buetler TM, Slone D, Eaton DL.* Comparison of the aflatoxin B 1 - 8,9–epoxide conjugating activities of two bacterially expressed alpha class glutathione S-transferase isozymes from mouse and rat. Biochem Biophys Res Commun 1992; 188(2): 597–603.
12. *Eaton DL, Gallagher EP.* Mechanisms of aflatoxin carcinogenesis. Annu Rev Pharmacol Toxicol 1994; 34: 135–72.
13. *Massey TE, Stewart RK, Daniels JM, Liu L.* Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B 1 carcinogenicity. Proc Soc Exp Biol Med 1995; 208(3): 213–27.
14. *Žikić RV, Štajn AŠ, Saičić ZS, Spasić MB, Milovanović SR.* Toxicologic significance of protection from oxidative damages. Kragujevac: Prirodno-matematički fakultet; 2000. p. 1–150. (in Serbian)
15. *Satoh K, Kitahara A, Soma Y, Inaba Y, Hatayama I, Sato K.* Purification, induction, and distribution of placental glutathione transferase: a new marker enzyme for preneoplastic cells in the rat chemical hepatocarcinogenesis. Proc Natl Acad Sci USA 1985; 82(12): : 3964–8.
16. *Alison MR.* Regulation of hepatic growth. Physiol Rev 1986; 66(3): 499–541.
17. *Habig WH, Pabst MJ, Jakoby WB.* Glutathione S-transferases. The first enyzmatic step in mercapturic acid formation. J Biol Chem 1974; 249(22): 7130–9.
18. *Lowry OH, Rosebrough NJ, Farr AL, Randall JR.* Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 195: 265–75.
19. *Hoel PG.* Introduction to mathematical statistics. New York: Willey; 1966. p.402–3.
20. *Rizvi TA, Mathur M, Nayak NC.* Enhancement of aflatoxin B 1 – induced hepatocarcinogenesis in rats by partial hepatectomy. Virchows Arch B Cell Pathol Incl Mol Pathol 1989; 56(5): 345–50.
21. *McMahon G, Davis E, Wogan GN.* Characterization of c-Ki-ras oncogene alleles by direct sequencing of enzymatically amplified DNA from carcinogen-induced tumors. Proc Natl Acad Sci USA 1987; 84(14): 4974–8.
22. *Muntanjola-Cvetković M.* Comprehensive Mycology. Beograd: Naučna knjiga; 1990. p. 259–62. (in Serbian)
23. *Wogan GN.* Aflatoxins as risk factors for hepatocellular carcinoma in humans. Cancer Res 1992; 52(7 Suppl): 2114s–8s.
24. *Allameh A, Farahani M, Zarghi A.* Kinetic studies of aflatoxin B 1 -glutathione conjugate formation in liver and kidneys of adult and weanling rats. Mech Ageing Dev 2000; 115(1-2): 73–83.
25. *Esaki H, Kumagai S.* Glutathione-S-transferase activity toward aflatoxin epoxide in livers of mastomys and other rodents. Toxicon 2002; 40(7): 941–5.
26. *Behroozikha M, Saidee M, Allameh A.* Comparison of aflatoxin B 1-DNA binding and glutathione conjugate formation by liver preparations from rats of different ages. Cancer Lett 1992; 66(1): 69–76.
27. *Wang C, Bammler TK, Guo Y, Kelly EJ, Eaton DL.* Mu-class GSTs are responsible for aflatoxin B 1-8, 9-epoxide-conjugating activity in the nonhuman primate macaca fascicularis liver. Toxicol Sci 2000; 56(1): : 26–36.
28. *Slone DH, Gallagher EP, Ramsdell HS, Rettie AE, Stapleton PL, Berlad LG,* et al. Human variability in hepatic glutathione S-transferase-mediated conjugation of aflatoxin B 1-epoxide and other substrates. Pharmacogenetics 1995; 5(4): 224–33.
29. *Goplan-Kriczky P, Hiruma S, Lotlikar PD.* Effect of glutathione levels on aflatoxin B_1-DNA binding in livers and kidneys of male rats and hamsters pretreated with buthionine sulfoximine and dimethylmaleate. Cancer Lett 1994; 76(1): 25–30.
30. *Liu YP, Lin Y, Ng ML.* Immunochemical and genetic analysis of the p53 gene in liver preneoplastic nodules from aflatoxin-induced rats in one year. Ann Acad Med Singapore 1996; 25(1): 31–6.
31. *Rogers AE, Kula NS, Newberne PM.* Absence of an effect of partial hepatectomy on aflatoxin B 1 carcinogenesis. Cancer Res 1971; 31(5): 491–5.
32. *Guerre P, Eeckhoutte C, Larrieu G, Burgat V, Galtier P.* Dose-related effect of aflatoxin B 1 on liver drug metabolizing enzymes in rabbit. Toxicology 1996; 108(1–2): 39–48.
33. *Raisuddin S, Parmar D, Zaidi SI, Singh KP, Verma AS, Seth PK,* et al. Aflatoxin induces depletion of activities of phase I biotransformation enzymes in growing rats. Eur J Drug Metab Pharmacokinet 1994; 19(2): 163–8.
34. *Wang CJ, Hsu JD, Lin JK.* Suppression of aflatoxin B 1-induced hepatoxic lesions by crocetin (a natural carotenoid). Carcinogenesis 1991; 12(10): 1807–10.

The paper was received on November 1, 2002.

Apstrakt

Strelić NJ, Saičić ZS, Magić ZM, Spasić MB, Trutić NV, Krtolica KV. Vojnosanit Pregl 2003; 60(4): 415–420.

PROMENE U AKTIVNOSTI GLUTATION-S-TRANSFERAZE U PLAZMI I JETRI PACOVA INDUKOVANE AFLATOKSINOM B_1

Uvod. Ispitivan je uticaj niskih doza aflatoksina B_1 (AFB_1) i parcijalne hepatektomije (PH) na aktivnost glutation-S-transferaze (GST) u plazmi i jetri pacova. **Metode.** Životinje su podeljene u četiri grupe. Prva (I) i druga (II) grupa su tretirane AFB_1 prethodno sveže rastvaranim u dimetilsulfoksidu (DMSO) i davanim u jednoj intraperitonealnoj dozi od 50 μg po pacovu (24 časa posle izvršene lažne operacije ili 40% PH). Treća grupa (III) bila je tretirana ukupnom dozom od 1 mg AFB_1, pet puta nedeljno tokom osam nedelja. Životinje koje nisu tretirane predstavljale su kontrolnu grupu (C). **Rezultati.** Značajno povećanje GST aktivnosti ($p<0,02 – p<0,005$) u poređenju sa kontrolnom grupom, utvrđeno je i u plazmi i u jetri svih ispitivanih grupa pacova. **Zaključak.** Dobijeni rezultati pokazuju da jednokratna, kao i višekratna primena niskih doza AFB_1 dovodi do dugotrajne indukcije aktivnosti GST u plazmi i jetri pacova, te da parcijalna hepatektomija nema veći uticaj na ovaj fenomen.

Ključne reči: **aflatoksini; karcinogeni; glutation transferaze; jetra; plazma; pacovi.**

Correspondence to: Nataša Strelić, Vojnomedicinska akademija, Institut za medicinska istraživanja, 11040 Beograd, Srbija i Crna Gora, Phone +381 11 662 722 Ext. 31 501