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Failure of oral gossypol to inhibit hepatic microsomal and cytosolic drug-metabolising enzymes

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Gossypol, a polyphenolic extract from the cotton seed and well known for its antifertility action, is also known to possess hypolipidemic, antitumor and antimicrobial properties [1]. Enzyme inactivation seems to be one of the major actions in bringing about its effect. Several key enzymes are known to be inhibited by gossypol, including the drug-metabolising enzymes [2, 3]. However, the inhibitory effects of gossypol on succinic acid dehydrogenase, cyto-

chrome oxidase and xanthine oxidase, observed *in vitro*, could not be demonstrated *in vivo* [4, 5]. Studies on the *in vivo* effect of gossypol on drug-metabolising enzymes are few and far between. The present study is an attempt to evaluate the effect of orally fed gossypol on the hepatic microsomal and cytosolic drug-metabolising enzymes in experimental rats. This forms a part of a major investigation undertaken to determine the underlying mechanism of gossypol-induced infertility [6]. Gossypol was administered orally at 20 and 30 mg per kg body weight for a period of 5–7 weeks, and the changes in hepatic aryl hydrocarbon hydroxylase (AHH), cytochrome P_{450}, microsomal UDP glucuronyl transferase (UDPGT) and cytosolic glutathione-*S*-transferase (GST) were studied.

Materials and methods

Male Wistar/NIN rats weighing around 250 g were randomly divided into five treatment groups as indicated in Table 1. Gossypol acetic acid was dissolved in a mixture of dimethyl sulfoxide (DMSO) and propylene glycol (PG) (1:9) and fed via an oesophageal tube. The control animals received an equal volume of DMSO–PG mixture, without gossypol acetic acid. Weekly records of body weights and food intake were maintained through the experimental period.

Rats were caged individually and maintained at 22 ± 2° with 12-hr light–dark cycles. A balanced stock colony diet along with water was fed *ad lib*.

Animals were killed at the end of the experimental period, and the liver and the testis from one side were excised and washed thoroughly. A 20% liver homogenate (w/v) in 0.154 M KCl in 0.01 M phosphate buffer, pH 7.4, was processed for the isolation of microsomes and the cytosol as reported earlier [7]. The microsomal mixed-function oxidase activity was estimated by measurements of cytochrome P-450 [8] and AHH [9]. The UDPGT was estimated using *p*-nitrophenol (0.4 mM) as the substrate in the presence of 2.5 mM uridine diphospho-5′-glucuronic acid as mentioned earlier [7]. GST was assayed with 1-chloro-2,4-dinitrobenzene (CDNB) according to Habig *et al.* [10] in the 105,000 *g* supernatant of liver and testis obtained from a 10% homogenate (w/v) in 0.25 M sucrose. Protein was estimated by the method of Lowry *et al.* [11]. Comparison between Groups I and II was done by Students *t*-test and between III, IV and V by analysis of variance [12].

Results and discussion

Oral administration of gossypol had no adverse effect on either the body weights or liver weights, as indicated in Table 1, at both dosages and durations. AHH was observed to be lowered by gossypol only at 30 mg/kg body weight at both time periods, though the difference at the end of 7 weeks was not significant, owing perhaps to the large variation. Cytochrome P-450, however, was not affected. In contrast, Xiao-Nan and Back [3] and Merril *et al.* [13] observed significant changes in cytochrome P-450 and mixed-function oxidases. Though Xiao-Nan and Back [3] observed a decrease in cytochrome P-450, the pharmacokinetics of tolbutamide requiring cytochrome P-450 for its metabolism was not affected. Furthermore, a close scrutiny of their data reveals that the observed changes in cytochrome P-450 are due to a generalised decrease in the microsomal protein content rather than to any specific effect. The microsomal protein content in our study was unaltered. The observations of Merril *et al.* [13] could possibly be due to the use of younger animals and the intraperitoneal route of administration of gossypol, thus indicating that the age and route of administration could be vital to the outcome of the effect of gossypol.

Hepatic UDPGT and GST in liver and testis, the major conjugating enzymes involved in xenobiotic detoxication, remained unaltered in the present study, although GST inhibition by gossypol has been demonstrated *in vitro* [14].

Table 1. Aryl hydrocarbon hydroxylase, cytochrome P-450, UDP glucuronyl transferase, and glutathione-S-transferase in gossypol-treated rats

Group	Gossypol acetic acid (mg/kg body wt.)	Duration in weeks	Body weight (g)		Liver wt (g)	AHH*	Cytochrome P-450†	UDPGT‡	Hepatic GST§	Testicular GST§
			Initial	Final						
I		5	264 ± 38	313 ± 26	10.9 ± 0.4	85 ± 17.6	0.52 ± 0.04	6.7 ± 2.4	1.4 ± 0.3	0.9 ± 0.2
II	30	5	242 ± 17	282 ± 16	10.9 ± 1.0	40 ± 17.3‖	0.64 ± 0.17	6.0 ± 1.2	1.2 ± 0.2	1.2 ± 0.4
III		7	249 ± 28	311 ± 33	11.0 ± 0.2	77 ± 23.6	0.50 ± 0.13	6.5 ± 2.9	1.3 ± 0.2	1.3 ± 0.2
IV	20	7	245 ± 22	298 ± 21	10.4 ± 0.7	62 ± 38.6	0.54 ± 0.10	5.4 ± 3.2	1.3 ± 0.4	1.2 ± 0.1
V	30	7	248 ± 25	294 ± 39	10.1 ± 0.7	56 ± 33.9	0.56 ± 0.22	5.6 ± 3.0	1.3 ± 0.3	1.1 ± 0.2

Values are means ± SD of four to six observations.
* Expressed in pmol of 3-OH BP formed per min per mg microsomal protein.
† Expressed in nmol per microsomal protein.
‡ Expressed in nmol p-nitrophenol conjugated per min per mg microsomal protein.
§ Expressed in μmol CDNB conjugated per min per mg cytosolic protein.
‖ Significantly different from Group I at $P < 0.05$.

Testicular lactate dehydrogenase-X, which is involved in sperm maturation and metabolism, also was not affected *in vivo* [6, 15] despite the demonstration of inhibition *in vitro*, by a number of workers [14, 16, 17], thus reaffirming the fact that gossypol has different effects *in vitro* and *in vivo*.

The significant feature of the present investigation is that the effect of oral gossypol on drug-metabolising enzymes was examined at the dosage regimens with demonstrated anti-fertility effect [6]. One of the possible explanations for the inconsistency between the *in vitro* and *in vivo* observations could be due to the occurrence of gossypol in a protein bound form, leading to inadequate levels of free gossypol to bring about inhibitory action. Working with isolated rabbit heart, Qian *et al.** observed the inhibition of the ventricular contractibility by gossypol in Lacke's solution but not in blood. Similarly, incubation with human serum albumin *in vitro* has been found to protect human and hamster lactate dehydrogenase-X from gossypol inhibition [18].

Based on these studies it can be safely concluded that gossypol fed orally over a period of 5–7 weeks at the dosage which produces infertility is unlikely to affect or inhibit xenobiotic detoxification.

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The effect of hyperthermia on conversion of rat hepatic xanthine dehydrogenase to xanthine oxidase*

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Interest in the potential of hyperthermia in the treatment of cancer stems from research demonstrating an increased sensitivity of cancerous cells to hyperthermia, compared to that of normal tissue [1, 2]. Hyperthermic liver perfusion has been utilized in attempts to treat patients with liver metastases arising from resectable colorectal cancer [3, 4]. One problem that appears to limit application of this technique, however, is the significant hepatotoxic effects of hyperthermic perfusion [4, 5].

Hepatotoxicity caused by hyperthermic perfusion is manifested by elevations in SGOT and LDH enzyme levels, and pathologically characterized by a centrolobular necrosis [4, 5]. We have suggested that this heat-induced toxicity is a consequence of oxidative stress, resulting in lipid peroxidative damage [5]. The process of lipid peroxidation is thought to be initiated by the reaction of an activated oxygen species with polyunsaturated fatty acids of cellular phospholipids, resulting in a chain-reaction formation of lipid hydroperoxides and aldehyde derivatives [6]. Lipid peroxidative processes and their biological consequences have been the subject of extensive research and numerous reviews [6–8].

Previous reports support the contention that hyperthermia results in oxidative stress within biological systems. Depletion of glutathione, an important cellular antioxidant, has been shown to increase the thermal sensitivity of cells in culture at 42–43° [9–13]. The redox state of hepatic cytoplasm, as measured by the lactate/pyruvate ratio, has been shown to be reduced markedly in both dogs and humans during hyperthermic perfusion, again with significant changes observed at 42–43° [14–16].

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