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Placental glutathione S-transferase (GST-P) induction as a potential mechanism for the anti-carcinogenic effect of the coffee-specific components cafestol and kahweol

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The coffee specific diterpenes cafestol and kahweol (C + K) have been reported to be anti-carcinogenic in several animal models. It has been postulated that this activity may be related to their ability to induce glutathione S-transferases (GSTs). We investigated the influence of a mixture of C + K, incorporated at various levels in the diet of Sprague–Dawley rats, on the expression of different hepatic GST iso-enzymes. Liver samples were examined using isoform-specific GST substrates and antibodies, and highly selective oligomers were employed to determine effects at the RNA level. A dose-dependent increase in general GST activity was observed in male and female animals following 28 or 90 days of treatment. A time-course study demonstrated that the maximal effect was observed within 5 days of treatment. Little or no effect was found on the activity of GST alpha and mu iso-enzymes. The most striking observation was a dose-dependent induction of placental glutathione S-transferase (GST-P) which could be demonstrated at the mRNA, protein and enzymatic levels. This effect was observed in both male and female rats. The maximal induction was attained within 5 days of treatment with C + K, remained elevated with continued treatment, but was reversible on withdrawal of treatment. Immunohistochemical examination of liver slices revealed a strong even distribution of GST-P expression throughout the acinus at the highest dose of C + K, while at lower doses the induction of GST-P occurred predominantly in periportal hepatocytes. There was no indication of the presence of preneoplastic foci and, furthermore, the effect of C + K on the GST-P was completely reversible. These findings indicate that the anticarcinogenic mechanism of C + K may involve a specific induction of GST-P and suggest a potential role for GST-P in detoxifying carcinogenic compounds.

Introduction

It has been recognized for a long time that diet may affect the development of cancer in laboratory animals as well as in man (1). Human foods contain a large number of well-characterized procarcinogens, both naturally occurring and also present as a result of contamination, some of which may have some relevance in this respect (2). However, an increasing body of evidence supported by epidemiological data, as well as experimental findings, indicates that in addition to carcinogens, the human diet contains a variety of chemical compounds which may possess protective, anticarcinogenic properties (3,4). These dietary anticarcinogens are very diverse in chemical structure and their mode of action is generally poorly understood (5). They may intervene at one or several steps of the carcinogenic process such as during the initiation, promotion or progression stages (5). It is well-documented that the initiation event for tumour formation, which generally consists of a permanent modification of DNA with electrophilic or oxidant metabolites derived from procarcinogen biotransformation, is a target for several dietary anticarcinogenic compounds, the so-called blocking agents (3,5). They act through an inhibition of the formation and/or the stimulation of the detoxification of the electrophilic or oxidant intermediates, resulting in decreased DNA damage and in the blocking of the initiation stage (3,5).

The glutathione S-transferases (GSTs*) are a family of multifunctional enzymes which catalyse the reaction of nucleophilic reduced glutathione with electrophilic compounds produced by phase I enzymes of the mixed function oxidising system (6,7). It has been postulated that GSTs may be involved in cancer chemoprevention by inactivating electrophilic proximate or ultimate carcinogens (5,7,8). Since induction of GST enzymes has been associated with protection against neoplasia (5,7–9), GST inducers are generally considered as protective compounds against cancer, acting as blocking agents (3,5).

The potential for a relationship between coffee consumption and cancer has attracted a lot of attention. In a review in 1991, the International Agency for Research on Cancer (10) reported that 11 out of 12 epidemiological studies have indicated an inverse relationship between coffee consumption and the risk of colon cancer and concluded that 'the collective evidence is compatible with a protective effect'. Several epidemiology studies published since 1991 have provided additional evidence supporting such an effect (11,12). For example, in a recent study performed in Sweden, a country characterized by a high coffee consumption, a dose-dependent pattern of findings compatible with a protective effect of coffee against colon cancer was clearly shown (12). However, there is insufficient data available to determine whether the chemoprotective effects of coffee are limited to colon cancer or if cancers at other sites are similarly affected. The general difficulty in discriminating protective effects of dietary components, as well as the particular problem of confounding factors associated with coffee consumption make data from such epidemiological studies very difficult to interpret.

Animal studies have provided evidence for a potentially more general chemoprotective effect of coffee and have led to the identification of some of the coffee components that may be responsible for these effects. For example, chronic exposure of rodents to instant coffee resulted in a decrease in the incidence of spontaneous tumours at a number of organ sites which could not be accounted for solely by an effect of

*Abbreviations: BPDE, (+)-7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene; C + K, cafestol and kahweol; DMBA, 7,12-dimethylbenz[*a*]anthracene; GST, glutathione S-transferases; GST-P, placental glutathione S-transferase; PhIP, 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine.

caffeine on calorie utilisation (13,14). Several studies have demonstrated that green as well as roasted coffees inhibit the development of 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced carcinogenesis at various tissue sites in different experimental animal cancer models (3,15–17). Subsequent investigations performed in rats and hamsters led to the identification of the lipidic diterpenes cafestol and kahweol (C+K) as two potentially chemoprotective agents in green coffee beans (18–20).

The mechanism responsible for the chemoprotective effects of C+K has not yet been elucidated. However, since C+K have been shown to induce GST activity (18,19), particularly GST mu (21), in mouse liver and small bowel, it has been hypothesized that these diterpenes may function as blocking agents. However, little is known about the effects of C+K on the expression of GSTs in rats or hamsters, the animal species in which the anticarcinogenic properties of these compounds have been demonstrated. In the present study, a combination of molecular, biochemical and enzymatic tools have been employed to analyse the isoform-specific expression of GSTs in rats fed a diet containing C+K. We have focused on the liver, the most important organ involved in carcinogen detoxification regardless of the eventual site of tumour development. For example, it has been illustrated for a number of diverse carcinogens that an increase in hepatic GST activity may induce a general protection state, leading to an inhibition in cancer initiation in the liver as well as in other sites such as mammary tissue (22). In contrast to the previous findings in mice, C+K administration did not result in an increase in GST mu in the rat liver. In this species, the major effect of C+K was a dramatic reversible, dose-dependent induction of the placental form GST-P.

Materials and methods

Treatment of animals

Twenty-one-day-old male and female Sprague–Dawley rats, obtained from Iffa Credo S.A. (l'Arbresle, France), were housed individually on dust-free wooden shavings in Macrolon cages maintained at 21–25°C and 45–65% relative humidity. The photoperiod was controlled to provide a 12 h light/dark cycle. The rats were fed laboratory chow (Nafag 890, Nähr und Futtermittel A.G. Gossau, Switzerland) as basal diet and were allowed free access to food and water at all times. The studies were performed under certified good laboratory practices (GLP).

In a first series of experiments, following 11 days of acclimatization on basal diet, the animals were randomly assigned to five treatment groups (A–E). Groups A and D were comprised of 15 male and 15 female animals each, while groups B and C contained 10 animals of each sex, and group E contained five animals of each sex. A mixture of C+K palmitates in a proportion of 52.5 and 47.5%, respectively, was solubilized at different concentrations in a 50:50 mixture of corn and palm oils employed as a vehicle. Group A received basal diet containing 2.5% of the vehicle. Groups B–E received basal diet with 2.5% of the vehicle containing increasing amounts of C+K to give final concentrations of C+K in the diet of: B = 92 ppm, C = 460 ppm, D = 2300 ppm, E = 6200 ppm. The mean daily intakes of the test product, calculated on the basis of weekly food consumption and body weight over a 90-day feeding period, were 7, 36 and 181 mg/kg body weight for males, and 7, 37 and 183 mg/kg body weight in female animals, respectively, for groups B, C and D. For group E animals, fed over a 28-day period, the mean daily intakes of test product were 717 and 672 mg/kg body weight for males and females, respectively. Subsets of animals (five males and five females per group) were killed after 28 days (groups A–E) or 90 days (groups A–D) of treatment. In addition, for a subset of groups A and D (five animals of each sex per group), a 1-month recovery period, in which animals were fed control diet, was employed after the 90-day test period in order to examine the reversibility of any effects. The animals were killed under anaesthesia by exsanguination following 20 h fasting, and the livers were excised and dissected into several portions. One piece was fixed in Bouin for immunohistochemical examinations, while others were immediately frozen in liquid nitrogen and stored at –80°C prior to being used for RNA isolation or for the preparation of cytosolic fractions.

In a second set of complementary experiments, the time-course for the effects of C+K was examined. Control (equivalent to group A of the first experiment) and test diet containing 2300 ppm of C+K (equivalent to group D) were administered to male rats. The animals were killed and liver samples taken for analysis (as described above) after 5 and 15 days on the test diets (five animals per group per time point). For the clarity of the data presentation, a zero-time point ($t = 0$), corresponding to the initiation of the study was derived from an average of values from control animals of the same sex, age and weight ($n = 11$).

Test material

A mixture of C+K palmitates was prepared from coffee oil according to the procedure of Bertholet (23). The mixture contained cafestol:kahweol in the proportions 52.5:47.5 and its purity was >95%.

RNA analysis

Total RNA was isolated according to the method of Chomczynski and Sacchi (24). The RNA concentrations were quantified by UV absorbance at 260 nm using an Uvikon 810 spectrophotometer (Kontron instruments). Dot blot analyses were performed according to standard procedures (25). Briefly, 5 µg of each RNA sample was applied to individual wells of a dot blot apparatus (96-well manifold system, Gibco BRL) mounted with nylon filters (Nytran N13). Following UV cross-linking, the filters were prehybridized, and then hybridized with a ^{32}P-labelled oligonucleotide complementary to GST Yp subunit mRNA (26). Northern analysis was performed to confirm the specificity of the hybridization. Relative mRNA contents were determined by scanning laser densitometry (Molecular Dynamics) of autoradiographs. The total RNA loading in each well was checked by subsequent hybridization using a ^{32}P-labelled oligonucleotide probe specific for 18S ribosomal RNA (27). The oligonucleotides used in the present study were obtained from Microsynth Gmbh (Balgach, Switzerland).

Protein analysis

SDS polyacrylamide gel electrophoresis (12% acrylamide) and Western blotting analysis were performed on the liver cytosolic fraction (10 µg per lane) according to standard methods (28). Detection was by enhanced chemiluminescence by the oxidation of luminol by horseradish peroxidase (ECL detection kit, Amersham Life Science) according to the protocol provided by the manufacturers. Relative Yp protein contents were determined by scanning laser densitometry (Molecular Dynamics). Polyclonal rabbit anti-rat GST Yp, Ya, Yc, Yb1 and Yb2 subunit antibodies were obtained from Biotrin International (Dublin, Ireland) and were used at a dilution of 1:1000. Protein concentrations were measured (29) using bovine serum albumin as the standard.

Enzymatic assays

GST activities of cytosolic fractions were assayed according to Habig *et al.* (30). 1-Chloro-2,4-dinitrobenzene (CDNB) was used as a substrate to measure general GST activity, while ethacrynic acid was employed as a 'specific' substrate for the analysis of the activity GST-P. The incubations were performed at 30°C.

Immunohistochemical analysis

Immunohistochemical reactions were performed on 2-mm thin paraffin-embedded liver sections mounted on slides pre-treated with poly-L-lysine (Sigma). The GST-P subunit expression was determined using the streptavidin biotin complex procedure (StreptABComplex/HRP, Dako). Polyclonal rabbit anti-rat GST Yp subunit antibodies (first antibody) obtained from Biotrin International (Dublin, Ireland) were used at a dilution of 1:2000.

Results

The test diets produced no abnormal behaviour and all animals remained in good health throughout the study period. In both male and female rats, treatment with C+K for 28 days produced a dose-dependent increase in liver GST general activity, which was significant ($P < 0.05$) at dose D (2300 ppm) and maximal at dose E (6200 ppm) (Figure 1A). A similar dose-response effect was found after 90 days of C+K treatment (data not shown). The time dependency of the C+K effect was evaluated using male rats treated at the dose D (2300 ppm) for 5, 15, 28 and 90 days. The increase in general GST activity occurred within 5 days and remained elevated at each of the measured time points (Figure 1B).

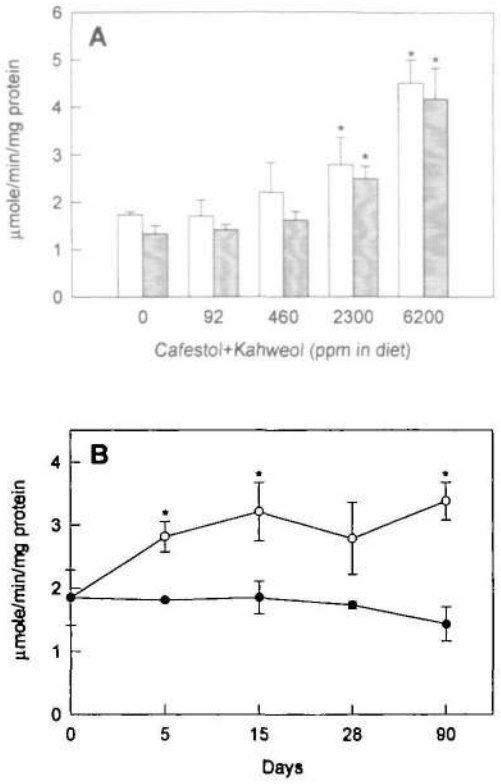


Fig. 1. (**A**) Dose-response effect of cafestol and kahweol (C+K) on general hepatic GST activity in Sprague–Dawley rats. The GST activity of liver cytosolic fractions of male (open bars) or female (hatched bars) rats fed either a control diet or diets containing C+K (92–6200 ppm) for 28 days was assayed using 1-chloro-2,4-dinitrobenzene as a substrate. (**B**) Time-course of the effects of C+K on general hepatic GST activity in rats. Male rats were fed *ad libitum* with control (closed circles) or test diet containing 2300 ppm of C+K (open circles). Values represent the mean ± standard deviation ($n = 5$). *Significantly different ($P < 0.05$) from animals fed control diet using Student's *t*-test.

GST isoform-specific molecular and biochemical probes were employed to identify which specific GSTs were responsible for the increase in general GST activity. The use of specific enzymatic substrates failed to reveal any significant alteration in GST alpha or GST mu dependent activities (data not shown). The absence of an effect of cafestol and kahweol on these GST families was confirmed at the protein level using Western blotting analysis. As shown in Figure 2, no differences in the expression of the GST sub-units Ya, Yc (GST alpha) and Yb1, Yb2 (GST mu) were observed in the liver of the C+K-treated animals as compared to the controls. The major effect produced by C+K was a dramatic induction of the expression and activity of the placental form GST-P as shown in Figure 3A. In both male and female rats, 28 days of treatment with C+K resulted in a dose-dependent increase in GST-P enzymatic activity which was significant ($P < 0.05$) at doses D (2300 ppm) and E (6200 ppm) for males, and at doses C (460 ppm), D (2300 ppm) and E (6200 ppm) for females. Treatment of the animals with C+K for 90 days resulted in a similar elevation of the GST-P enzymatic activity (data not shown). The results for the time-dependent effects of C+K on GST-P activity, presented in the Figure 3B, demonstrate that at dose D (2300 ppm), the maximal increase in GST-P activity was already apparent within 5 days of treatment with C+K and was maintained during the 90-day treatment period. Since ethacrynic acid is not strictly specific for GST-P, but is

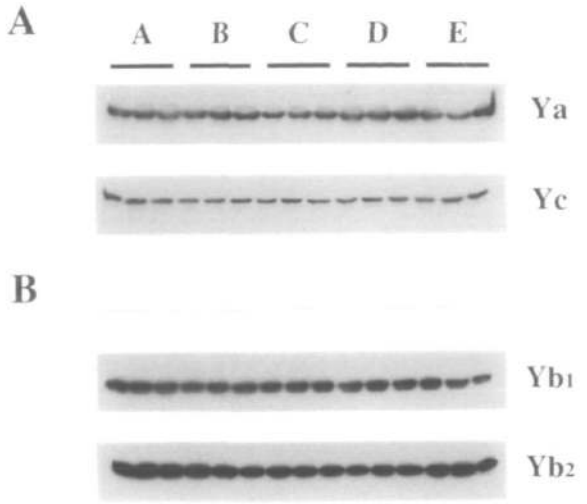


Fig. 2. Dose-response effect of cafestol and kahweol (C+K) on the expression of (**A**) hepatic GST alpha subunits Ya and Yb; and (**B**) GST mu sub-units Yb1 and Yb2, in Sprague–Dawley rats. Cytosolic fractions from livers of male rats fed either a control diet or diets containing C+K (92–6200 ppm) for 28 days were assayed for the expression of the various GST subunit proteins by Western blotting using specific antibodies. C+K (ppm in diet): A = 0; B = 92; C = 460; D = 2300; E = 6200.

also a substrate for other isoforms of GST, Western blotting using highly specific antibodies for GST Yp sub-unit was employed to confirm that the increased enzyme activity was correlated with a parallel elevation of the expression of GST Yp protein. For this purpose cytosolic fraction of samples from the 28-day dose-response study were analysed and representative results are provided in Figure 4. In control animals little signal specific for GST Yp protein was detectable. In both sexes a dose-dependent increase in the Yp protein expression, was clearly observed at doses C (460 ppm; 9-fold induction), D (2300 ppm; 14-fold induction) and E (6200 ppm; 14-fold induction) for males, and at doses D (2300 ppm; 4-fold induction) and E (6200 ppm; 7-fold induction) for females.

Northern blotting for mRNA using a highly specific oligomer cDNA for GST Yp was applied to a limited number of samples (males from the 28-day dose-response study) to optimize the hybridization conditions. No signal was detectable in the control animals while a single band corresponding to the expected size of the GST Yp subunit mRNA was clearly visible in samples corresponding to the two highest doses examined (data not shown). The effects of C+K on the expression of Yp mRNA were then quantitated by dot-blot analysis employing the optimised hybridisation conditions. Densitometric analysis of autoradiograms from the 28-day dose-response and the time-course studies are presented in Figure 5. C+K produced an induction of the expression of the Yp mRNA in both male (at 2300 and 6200 ppm) and female (at 6200 ppm) rats. Maximal induction was achieved within 5 days of treatment and was maintained during the whole treatment period (90 days).

The effects of C+K on the spatial pattern of GST-P expression within the liver was evaluated in immunohistochemical studies. Figure 6 illustrates the data obtained for male rats exposed to C+K for 28 days. In control animals, biliary duct epithelial cells stained positively as did a small number of individual hepatocytes. A dose-dependent increase in the immunostaining specific for GST Yp was apparent in the C+K treated animals. At doses C (460 ppm) and D (2300

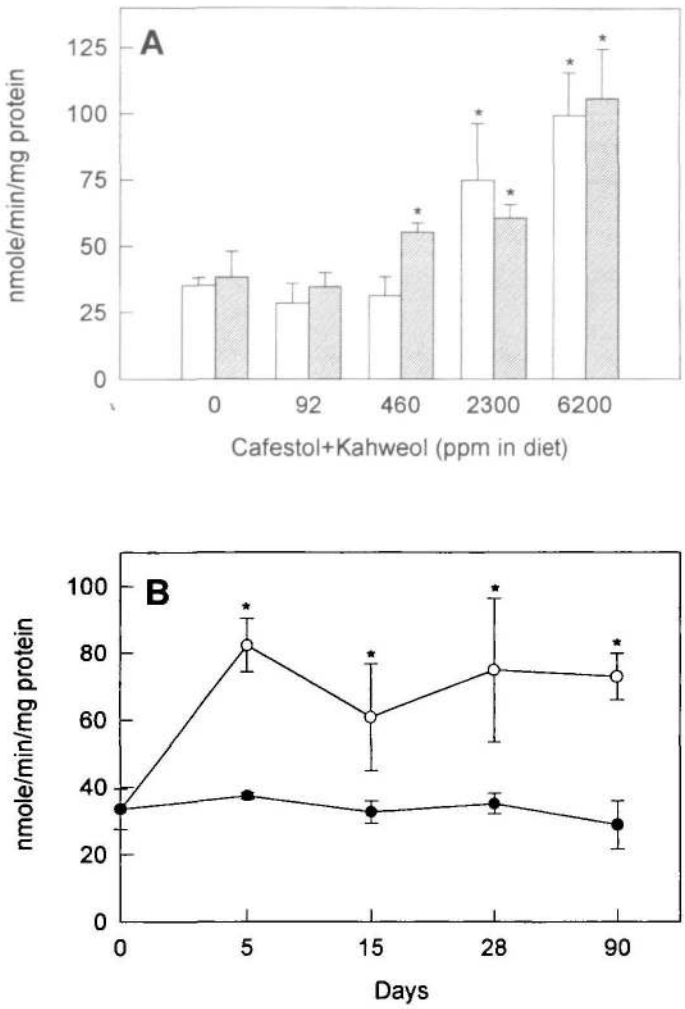


Fig. 3. (**A**) Dose-response effect of cafestol and kahweol (C+K) on the activity of hepatic GST-P in Sprague–Dawley rats. The GST-P activity of liver cytosolic fractions of male (open bars) or female (hatched bars) rats fed either a control diet or diets containing C+K (92–6200 ppm) for 28 days was assayed using ethacrynic acid as a specific substrate. (**B**) Time-course of the effects of C+K on hepatic GST-P activity in rats. Male rats were fed *ad libitum* with control (closed circles) or test diet containing 2300 ppm of C+K (open circles). Values represent the mean ± standard deviation ($n = 5$). *Significantly different ($P < 0.05$) from animals fed control diet using Student's *t*-test.

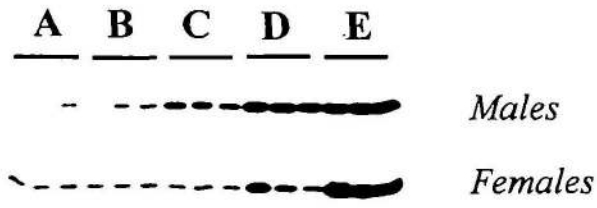


Fig. 4. Dose-response effect of cafestol and kahweol (C+K) on the expression of hepatic cytosolic GST Yp subunit protein in Sprague–Dawley rats. Cytosolic fractions from livers of male and female rats fed either a control diet or diets containing C+K (92–6200 ppm) for 28 days were assayed for the expression of GST Yp subunit protein by Western blotting using specific antibodies. C+K (ppm in diet): A = 0; B = 92; C = 460; D = 2300; E = 6200.

ppm) the staining was restricted to the periportal areas, while in the rats which received the highest C+K dose an homogenous immunostaining of the whole liver acinus was

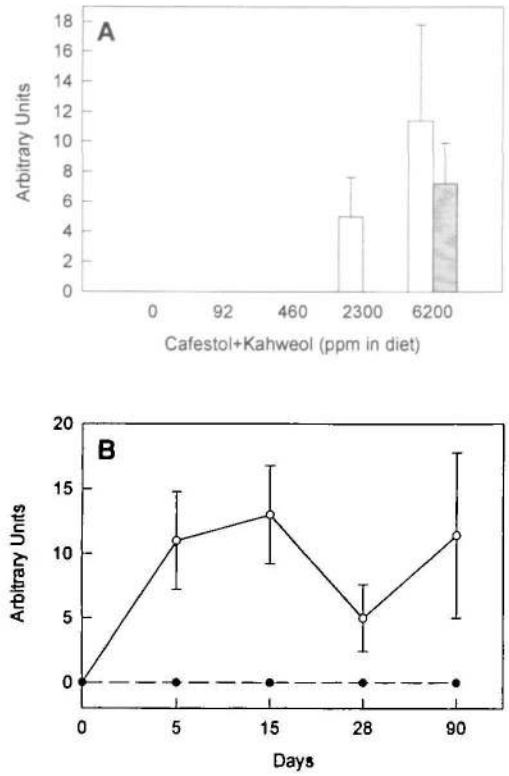


Fig. 5. (**A**) Dose-response effect of cafestol and kahweol (C+K) on the expression of hepatic GST Yp mRNA in Sprague–Dawley rats. Total RNA fractions extracted from livers of male (open bars) or female (hatched bars) rats fed either a control diet or diets containing C+K (92–6200 ppm) for 28 days were subjected to dot blot analysis using an oligomer specific for GST Yp mRNA. Relative mRNA contents were determined by scanning laser densitometry (Molecular Dynamics) of autoradiographs (**B**) Time-course of the effects of C+K on the expression of hepatic GST Yp mRNA in Sprague–Dawley in rats. Male rats were fed *ad libitum* with control (closed circles) or test diet containing 2300 ppm of C+K (open circles). No signal was detected for GST Yp mRNA in control samples. Values represent the mean ± standard deviation ($n = 4$).

apparent. Similar patterns of expression were observed after 90 days of C+K treatment and also in a less pronounced manner in female animals fed C+K for 28 or 90 days (data not shown). It should be emphasized that in both sexes there was no evidence of any GST-P focal staining in any of the samples examined in these experiments.

The reversibility of the C+K-induction of hepatic GST Yp expression was examined in male rats. As expected exposure to C+K (2300 ppm) for 90 days was characterized by a significant induction of GST-P as assessed by both enzymatic and Western blotting techniques. However, following the 1 month recovery period on control diet, the GST-P specific enzymatic activity (Figure 7A) as well as the Yp protein content (Figure 7B) decreased to similar levels as observed in animals which had been continually fed the control diet. The reversibility of the C+K induction of GST Yp was confirmed using immunohistochemical staining (data not shown).

Discussion

Several studies have demonstrated that green as well as roasted coffee inhibit the development of DMBA-induced carcinogenesis in different experimental animal cancer models (3,15–17). Subsequent investigations performed in rats and hamsters led to the identification of C+K as potential chemo-

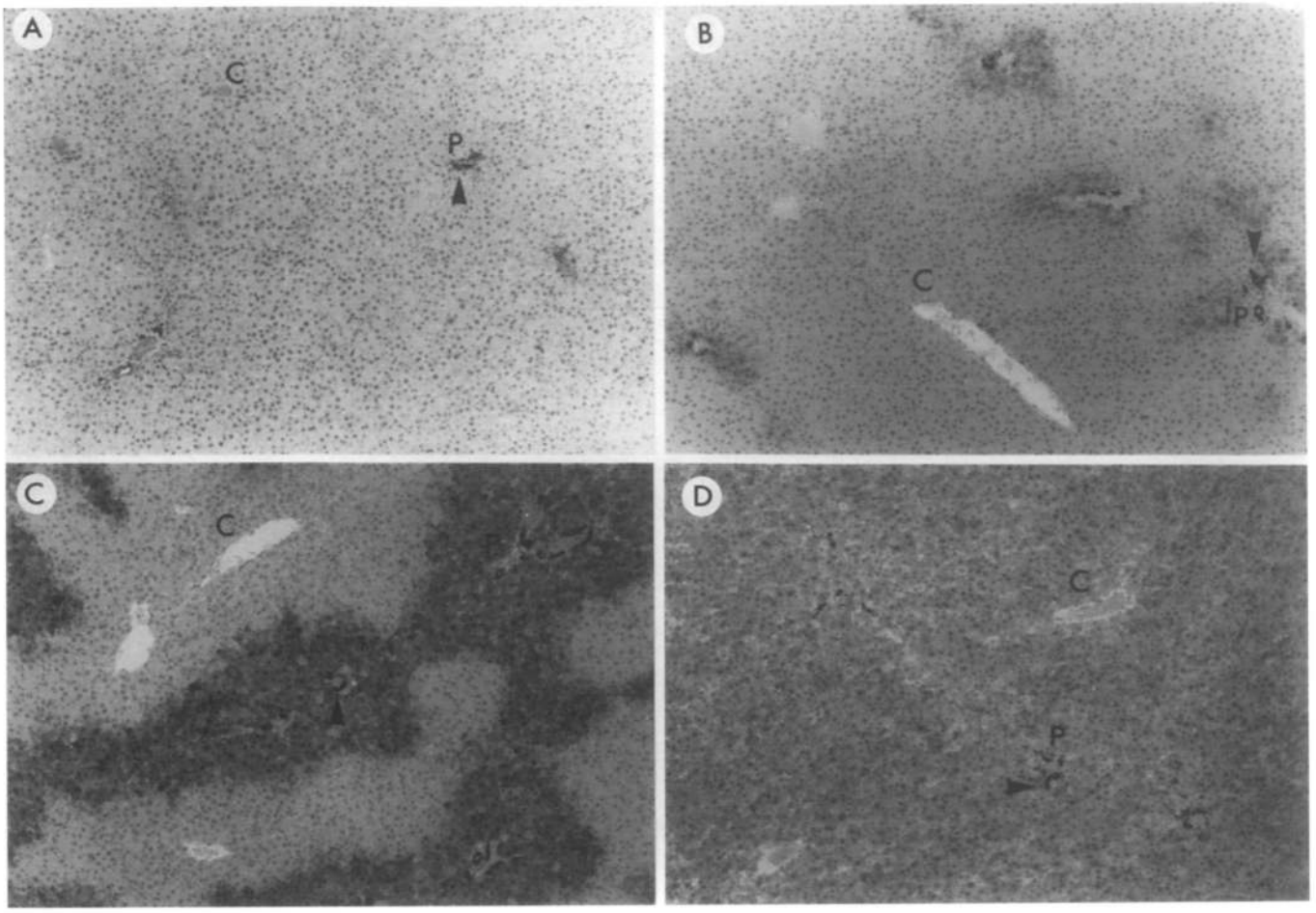
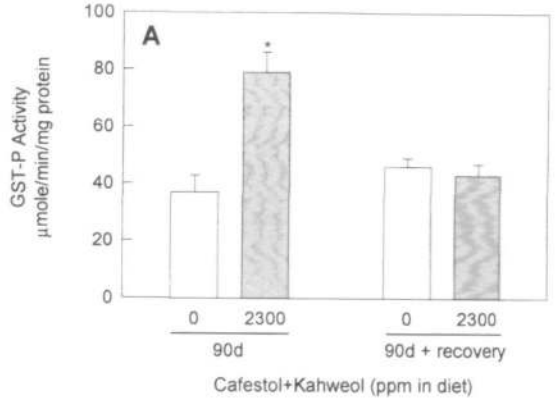


Fig. 6. Immunohistochemical localisation of GST Yp subunit expression in livers from male rats fed *ad libitum* with either a control diet or diets containing cafestol and kahweol (C+K) for 28 days. (**A**) Control diet; (**B**) 460 ppm C+K; (**C**) 2300 ppm C+K; (**D**) 6200 ppm C+K. Bile ducts are indicated by arrow heads. C = central vein, P = portal field. (Magnification ×25.)

protective agents (18,19). The mechanism(s) responsible for the chemoprotective effects of C+K have not been elucidated. However, since C+K have been shown to induce a GST mu-dependent enzymatic activity in mice (21), it has been proposed that C+K belong to the family of type A blocking agents and act through a stimulation of the detoxification processes supported by GST mu (21).

In the present investigation, studies were performed to characterize the potential effects of C+K on GST iso-enzyme expression in rats, one of the animal species in which the anticarcinogenic properties of these coffee components have been demonstrated (3,19). As observed in mice (18,19,21), a significant dose-dependent increase in the hepatic total GST activity was found in C+K treated rats. In contrast, a selective increase in GST mu-dependent activity was not detected. Rather, the major effect of C+K identified in the rat model was a specific induction in GST-P expression which paralleled the increase in total GST activity. Although this induction of GST-P was the most striking effect observed, we cannot eliminate the possibility that other GSTs, such as GST theta, which were not analyzed could also be involved in the overall effects of C+K on general GST activity. Nevertheless, the findings presented demonstrate a previously unreported species-specificity in hepatic C+K responsiveness. Since the molecular forms of GST expressed in the untreated mouse liver differ considerably from those found in rat liver (7,31,32) this species specificity is not surprising. Indeed, rat GST-P is expressed at a very low level in the adult liver (31) in contrast to its mouse equivalent GST MII, which is constitutively expressed in adult mouse liver (32). Furthermore, several other lines of evidence have demonstrated differences between these two rodent species regarding the regulation of other GST subunits (7). The finding of a species specificity in the control of GST subunit expression raises the question of the potential nature of the human response to C+K exposure since little is known about which animal model most closely reflects the situation in man.

In rats, the placental GST Yp subunit is expressed in many normal extra-hepatic tissues as well as in foetal liver (6,7,31). However, it is only barely detectable in the adult liver (6,7,31). This particular GST iso-enzyme has attracted a lot of attention over the past decade since it has been proposed as a reliable marker of preneoplastic and neoplastic lesions (33). It is well documented that profound permanent biochemical changes occur during rat hepatocarcinogenesis and with respect to xenobiotic metabolism the expression of phase I enzymes is often depressed while those of the phase II enzymes is increased (34). In preneoplastic and neoplastic hepatic tissue, the alpha, mu and pi classes of GST have been reported to be elevated (6,7,35,36). Of these, GST-P is considered the most promising marker of (pre)neoplastic lesions. It is constitutively and irreversibly expressed in most initiated hepatocytes, preneoplastic foci, hepatocellular nodules and hepatocarcinomas (6,7,33) and its up-regulation can be observed following



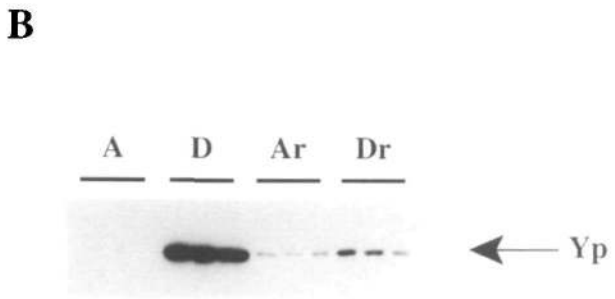


Fig. 7. (**A**) Reversibility of the effects of cafestol and kahweol (C+K) on hepatic cytosolic GST-P activity. Male rats were provided with control diet (*open bars*) or diet containing 2300 ppm C+K (*hatched bars*) for 90 days. For a subset fed either diet, a one month recovery period in which animals were fed control diet was employed after the 90-day test period in order to examine the reversibility of any effects. Values represent the mean ± standard deviation ($n = 5$). *Significantly different ($P < 0.05$) from animals fed control diet using Student's-test. (**B**) Reversibility of the effects of cafestol and kahweol (C+K) on the expression of hepatic cytosolic GST Yp subunit protein as determined by Western blotting using specific antibodies. Male rats were provided with control diet (A) or diet containing 2300 ppm C+K (D) for 90 days. For a subset fed either diet (Ar and Dr), a 1-month recovery period in which animals were fed control diet was employed after the 90-day test period in order to examine the reversibility of any effects.

exposure to numerous different hepatocarcinogenic compounds (37). Furthermore, expression of this enzyme in (pre)neoplastic cells, but not in surrounding hepatocytes greatly facilitates the immunohistochemical detection of the lesions. Consequently, GST-P has been extensively used to study the development of the carcinogenic processes and has provided the basis for new methods of screening for carcinogens and carcinogenic modifiers (37). In the present study a substantial induction of GST Yp protein associated with a concomitant increase in its enzymatic activity was observed in the liver of rats fed a diet containing C+K. The parallel increase in the levels of the specific Yp mRNA observed at the highest C+K doses suggests a transcriptional activation of the *GST-P* gene and/or a specific increase in Yp mRNA stability. Northern as well as slot blotting analysis failed to reveal a detectable mRNA signal at doses (C for males, D for females) at which a significant increase in the Yp sub-unit expression was clearly identified at the protein level. This apparent paradox reflects the differential sensitivities of the two assays and highlights the relatively high sensitivity of Western analysis for GSTs. Immunohistochemical examinations revealed that the highest dose of C+K induced a homogenous expression of GST Yp throughout the whole liver acinus, while the lowest active doses of C+K induced GST Yp predominantly in periportal areas. Even after 90 days of continuous treatment with C+K there was no evidence of GST Yp focal staining. Furthermore, a recovery study demonstrated that the increase in GST-P expression was dependent on the continuous presence of the test compound in the diet and was reversible following removal of C+K. When considered in association with the finding that the C+K-dependent effect on GST-P occurs within a short time period (≤5 days), these results indicate that the effect of C+K on this GST isoform is a transient induction which is unrelated to the permanent derepression of GST-P which occurs as a result of cell transformation.

A number of other dietary constituents with chemoprotective properties have been shown to produce similar effects on the expression of GST-P. For example, administration of ethoxyquin to rats has been shown to increase the hepatic expression of GST-P in the liver (38), predominantly in portal areas (39). Furthermore, the two commonly used food antioxidants butylated hydroxytoluene and butylated hydroxyanisole (40), a naturally occurring flavone (41), as well as dithiolethiones (42) were shown to significantly increase the expression of GST-P in the rat liver. It thus appears that GST-P is inducible through the action of various dietary agents possessing anticarcinogenic properties in animal models. Interestingly, several of these compounds, such as ethoxyquin and butylated hydroxytoluene (43), have been associated with protection against DMBA-induced mammary cancer. An important biotransformation pathway of DMBA is glutathione conjugation although the identity of the GST subunits involved has not been completely established (44). Taken together these data suggest that GST pi may be involved in the detoxification of this carcinogen.

Several lines of evidence indicate that GST pi may play a significant role in chemoprotection. Human as well as rat GST pi have both been reported to detoxify carcinogenic compounds. Rat hepatoma cell lines expressing high levels of GST-P were shown to efficiently conjugate and detoxify the highly mutagenic and carcinogenic compound (+)-7β,8α-dihydroxy-9α, 10α-oxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE) (45). Similarly, the transfection of cells with the human GST pi cDNA was characterized by the induction of a protection against the alkylation of macromolecules by the carcinogen 4-nitroquinoline-1-oxide (NQO) (46). Furthermore, these recombinant cells showed increased resistance to toxicity induced by benzo[*a*]pyrene-(anti)-7,8-dihydrodiol-9-10-epoxide (46). *In vitro* studies have demonstrated that human GST pi possesses some capacity to inhibit the covalent DNA binding of the N-acetoxy derivative of 2-amino-1-methyl-6-phenylimidazo(4,5-*b*)pyridine (PhIP) the ultimate carcinogenic metabolite of the food-borne carcinogen PhIP (47). In addition, a role for GST pi in the detoxification of highly toxic electrophilic unsaturated carbonyl compounds produced by radical reactions, lipid peroxidation and ionising radiations has recently been established (48,49).

Thus, the anaysis of data from a number of previous studies strongly suggests that GST-P plays an important role in the biotransformation of certain carcinogenic and/or toxic substances. In addition, it is well documented that a stimulation of hepatic detoxification processes may significantly alter cancer development at extra-hepatic sites. For example, some protective effects of butylated hydroxytoluene against DMBA-induced mammary tumours have been associated with an induction of hepatic GST activity in the absence of a similar effect in mammary tissue (22). Thus the finding reported in

the present study, namely that GST-P is strongly induced by cafestol and kahweol in rat liver, provides a potential mechanism to support the previous findings that these natural coffee components possess chemoprotective properties.

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