Research Article

Bioactivation of Herbal and Dietary Constituents of Herbal Medicines and Normal Diet may lead to Toxicological Outcomes in Humans

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ABSTRACT

The growing popularityof herbal and dietary constituents use to improve healthcalls for critical appraisal of the "natural" equal "safe" perception among consumers. Phytochemicals may undergo bioactivation by Phase I or Phase II xenobiotic metabolizing enzymes and potentially lead to deleterious effects. Various phytotoxin-induced organ damages have been previously described. In this review, phytochemicals which are commonly part of herbal medicines and normal diet were examined and used to highlight possible toxicological risk via bioactivation.This relates to compounds such as flavonoids,safrole, methyleugenol, estragole, coumarin, aristolochic acid and cyanogenic glycosides. It is concluded that possible toxicity risks exist via metabolic activation although overall manifestation is multifaceted and depends on interplay of factors.

1. INTRODUCTION

Interest for plant-based ingredients and their use as food supplements or other preparations to treat various ailments and to improve health in general continues to rise globally. This rise is, partly, grounded in the perceptions of relative safety because their natural origin andlong-use in folk medicines.Additionally, there seems to be a lax worldwide among regulation agencies in subjecting natural-based products to the scrutiny of the approval process applied to new drug applications. In spite of the fact that some plant-based ingredients are present in fruits and vegetables components of our diet, they are generally regarded as safe (GRAS) at their exposure levels. However, toxicological concerns may arise at elevated levels of exposure.Available literature evidence indicates that phytochemicals may undergo metabolic activation to reactive metabolites with ability to potentially modify various cellular macromolecules via covalent binding leading to toxic outcomes[35]. This review describes metabolic activation of some selected phytochemicals as important herbal and dietary constituents to assess possible toxicological risks. The herbal and dietary constituents chosen have well-known potential toxicities but are part of normal diets or herbal medicines. This includesflavonoids,safrole, estragole and methyleugenol, coumarin, aristolochic acid and cyanogenic glycosides.

2. Biotransformation enzymes and xenobiotics

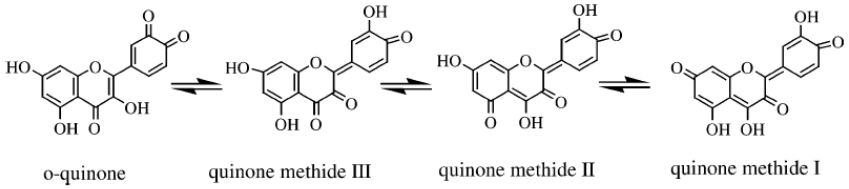
The major physiological function of biotransformation enzymes in the human body is to convert xenobiotics into readily excretable hydrophilic metabolites in order to maintain homeostasis. Biotransformation typically occurs in two distinct phases. Phase I leads to introduction of a functional group either by insertion or unmasking[32]. Phase II enzymes catalyze conjugation with endogenous substratesto form highly hydrophilic metabolites.The primary roles of xenobiotic metabolizing enzymes are detoxification and elimination of chemicals. However, metabolism may also generated toxic metabolites. Literature evidence clearly point to the fact that metabolism might result in activation of an innocuous substance to reactive, toxic metabolites[32]. Such reactive electrophiles are capable of modifying vital cellular macromolecules such as DNA and proteins via covalently bonding leading to different forms of toxicity. Toxicity may result indirectly from interactionsof reactive metabolites with molecular oxygen to generate reactive oxygen species[32]. Potential toxicities of some plant-based ingredients which are commonly present in fruits and vegetables components of our diet are illustrated in the subsequent sections. Also, factors which may influence the expression of such intrinsic toxicities are discussed.

3. Flavonoids

The natural polyphenol quercetin is an abundant and widely studied flavonoid. Generally, flavonoids are important constituents of fruits, vegetables, nuts, seeds, tea, olive oil and red wine [47;51]. The protective effects of flavonoids may result from their anti-oxidative properties[9;47;51]. Flavonoids likely modulate the metabolism of food-born carcinogens throughinhibition and/or induction of phase I and II biotransformation enzymes. Such modulations have been implicated in other health benefits such as prevention of carcinogenesis [7;19;34;51]. Notwithstanding the scientific support for the various health claims, important toxicological concerns mainly due to toxic pro-oxidant formation at higher doses have been reported[14].

3.1. Bioactivation and related toxicities

The quinone/quinone methide chemistry of quercetin may be related to its mutagenic properties[14,48]. Metabolic activation of quercetin involves enzymatic oxidation to quercetin ortho-quinone, followed by isomerisation to quinone methides*(Scheme 4)*[11]. These quinone methides are active alkylating DNA-reactive intermediates.

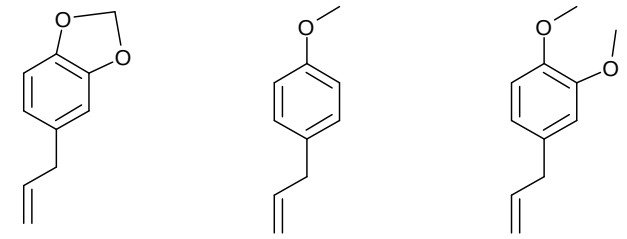


Scheme 4: Quinone/quinone methide isomerisation of quercetin[11]

The quinone type metabolites of quercetin and other flavonoids appear to be highly unstable and only formed in vitro as adducts using trapping agents like gluthathione.[5, 11]. This transient nature of the covalent quercetin adducts likely have consequences for extrapolation of genotoxicity to carcinogenicity in vivo. There seems to be scientific consensus on the absence of tumour initiation by quercetin[33;53;73]though few studies reported contrary findings[20;56]. The transient nature of quercetin quinone methide adducts have been cited as a possible explanation of why genotoxic characteristics of quercetin are not resulting in carcinogenicity[77]. The relevance for the human in vivo situation remains unstudied.

4. Safrole, Estragole and Methyleugenol

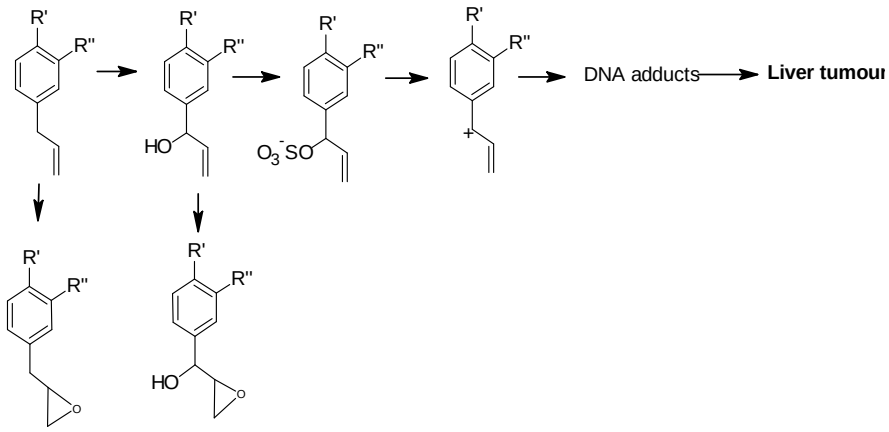
The herb-base ingredients safrole, estragole and methyleugenol (*Scheme 1*) are structurally related compounds of the chemical class alkenylbenzenes. These compounds occur naturally in nutmeg, mace, cinnamon, tarragon, basil, anise, black pepper, lemongrass fennel among others[22-24]. These herbs and spices have been used for culinary and medical purposes throughout the world.



Scheme. 1: Structural formulas of Safrole, Estragole and Methyleugenol

4.1 Bioactivation and related toxicities

Alkenylbenzenes are bioactivated by different CYP enzymes to the major proximate carcinogen, 1-hydroxymetabolites. In particular, CYP 1A2 and 2A6 enzymes are responsible for the formation 1-hydroxymetabolites[59]. Sulfotransferases (SULF) enzymes activities on 1-hydroxymetabolites generate electrophilic species (Scheme 2) which are implicated in their toxicities. The contributions of other metabolic activation routes as well as the parent compounds to the overall toxicity seem to be species dependent. Such differences may result from interspecies differences in drug metabolizing enzymes.



Scheme 2: Bioactivation pathways of alkenylbenzenes

4.1.1 Carcinogenicity

Safrole

Safrole and its 1-hydroxysafrole metabolite showhepatocarcinogenic effect in mice and rats although the latter shows higher toxicity[49]. Mice but not rats are susceptible to 1-hydrosafrole-2,3-oxide induced-hepatic carcinomas[49].The significant inhibition of hepatomas formation after treatment with pentachlorophenol of experimental animals suggests that carcinogenesis is mediated by sulfooxysafrole metabolites[78]. In another study, DNA adduct formation of 1-hydroxyfrole was inhibited in theliver of mice deficient in synthesis of PAPS, the cofactorrequired for sulfotransferase reactions[10].

Methyleugenol

Induction of liver tumours by methyleugenol and its proximate carcinogenic metabolite 1-hydroxymethyleugenol have been demonstrated in mice and rats[48;54]Available literature evidence suggests methyleugenol is a multisite and multispecies carcinogen[54].

Estragole

Estragole, 1-hydroxyestragole, estragole-2,3-oxide and 1-hydroxyestragole-2,3-oxide have been shown to induce hepatic tumours in mice[50;62]. Finding of inhibition studies with pentachlorophenol supports involvement of sulfooxyestragole metabolites in estragole-induced cancers[46]. In addition, Miller et al.[49] found a significant increase in lung adenomas after exposing mice to 1-hydroxyestragole-2,3-oxide. Except for one study which reported no significant increases in hepatic carcinomas in male Fischer rats[52], carcinogenicity of estragole and its metabolites remains unstudied in rats.

4.1.2 Genotoxicity

Safrole

In a regulatory standard test for genotoxicity alkenylbenzenes exhibited a strong potential for DNA damage[34] Howes et al.[30] reported a dose-related increase in UDS response in mice and rat hepatocytes after exposure to safrole. The responses were however higher in mice than were seen in rat hepatocytes. The major adduct isolated from mouse liver was 1-hydroxysafrole adducts of guanine[57].The DNA adducts of safrole-2,3-oxide metabolite or safrole were only detected in vitro[58]indicating that these may not be connected with in vivo genotoxic properties. In coincubation assays, the sulfotransferase inhibitor pentachlorophenol inhibited UDS responses[16] which supports electrophilic carbocation mediated pathway in genotoxicity. Another study corroborated this finding when it found that pentachlorophenol prevented the binding of safrole with mouse liver DNA[61].

Methyleugenol

UDS testhas shown that methyleugenol as well as its metabolites 1-hydroxymethyleugenol and methyleugenol-2, 3-oxide is genotoxic in cultured rat hepatocytes in vitro[34]. Like safrole, methyleugenol induced higher UDS responses in mice hepatocytes than were seen in rats hepatocytes andUDS responses were inhibited by pentachlorophenol in coincubation assays[15]. In addition, guanine adducts of 1-methyleugenol was the major DNA adduct formed in mice liver tissue[57].

Estragole

Estragole and its metabolites showed genotoxic effects in UDS test. The 1-hydroxyestragole was shown to be the major

proximate carcinogen. DNA adducts of estragole have also been studied in mice and like safrole the major DNA adduct was guanine adduct of 1-hydroxyestragole. In addition, estragole-2,3-oxide and 1-hydroxyestragole-2,3-oxide produced DNA adducts in vitro[29;59]. However, adducts of these epoxides were not detected in mouse liver following in vivo administration of estragole[45]. This suggests that estragole epoxidation may not contribute to genotoxicity and has been ascribed to very rapid and efficient detoxification of the 2,3-oxides in the cell by a combination of epoxide hydrolases and glutathione S-transferases[28].

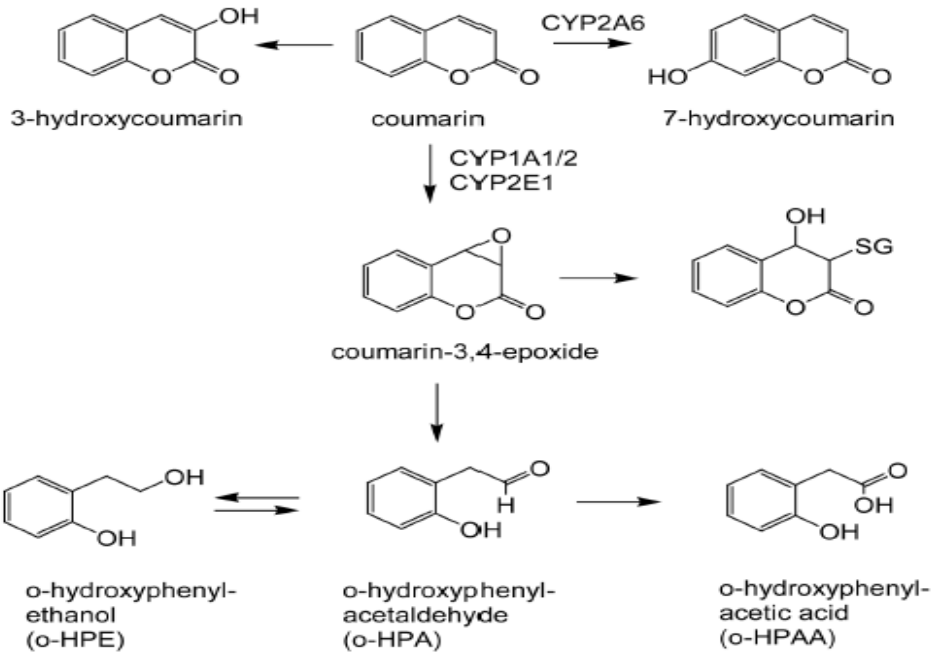
In summary,cytochrome P450 and sulfotransferase catalyzed bioactivation pathways leading to 1-hydroxymetabolites and 1-sulfooxymetabolites mediate carcinogenicity and genotoxicity of the alkenylbenzenes[60]. The alkenylbenzenes exert their major carcinogenic effect in the liver. The hepatocarcinogenicity proceeds mainly via the 1-hydroxymetabolite, but also estragole-2,3-oxide and 1-hydroxysafrole-2,3-oxide has been shown to cause hepatocarcinogenic effects in some studies. The epoxide from the parent compounds or their 1-hydroxymetabolites are to some extent involved in the formation of lung and skin carcinoma. The organ specific expression of the P450 enzymes involved in the bioactivation of alkenylbenzenes may likely relate to the organ specific toxicities.

5. Coumarin

Coumarin is present at high levels in some essential oils such as cinnamon leaf oil, lavender oil, peppermint oil and is also found in fruits, green tea and in personal care products[39].In 1954, coumarin was banned from use as food flavours in the US and later in the UK after hepatotoxic effects were reported in rats and dogs[38;69]

5.1 Bioactivation and related toxicities

Chronic exposure to coumarin leads to liver adenomas and carcinomas in rats and liver adenomas in mice[21;40]. Induction of liver tumours is thought to proceed by non-genotoxic mode[21] because no covalent binding of coumarin to DNA were identified in kidney and liver of coumarin-exposed rats. Coumarin undergoes CYP enzymes dependent 3,4-epoxidation to generate reactive coumarin epoxide. The epoxide may rearrange spontaneously to the hepatotoxic intermediate o-hydroxyphenylacetaldehyde (o-HPA) (Scheme 5)[41;43]. However, bioactivation of coumarin via 3,4-epoxidation is reported to be significantly species dependent. Although 3,4-epoxidation pathway appears to be the major route of coumarin biotransformation in rats and mice, it is not the case in humans. In humans, a CYP 2A6 mediated detoxification to 7-hydroxycoumarin was found to be the major route[13;26;37]. Also, the hepatotoxic intermediate o-HPA was more efficiently detoxified in humans than in rats[75].



Scheme 5: Biotransformation of coumarin leading to detoxification and bioactivation[67]

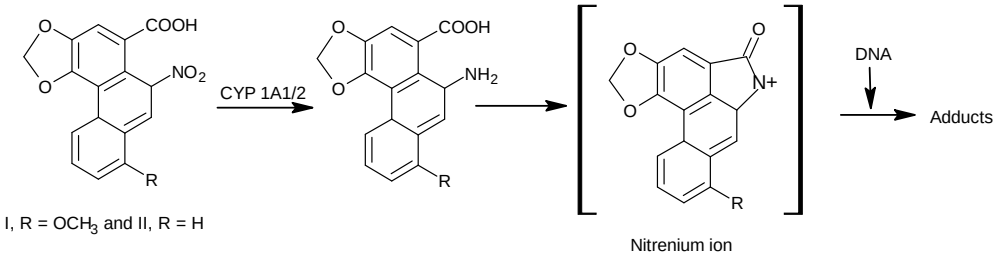
There is little evidence of coumarin-induced toxicity in humans. However, genetic polymorphism in human CYP2A6 appears to cause marked interindividual variation in coumarin metabolism to 7-hydroxycoumarin in humans[42;66]. The role of CYP2A6 polymorphism in human risk profiles for coumarin has not been well studied.

6. Aristolochic Acid (AA)

The plant *Aristolociaceae* has long history of use in herb-based medicines. The aristolochic acids I and II *(Scheme 6)* which are constituents of this plant are nephrotoxic, genotoxic, and carcinogenic[4;6;68]. After the first reported case of kidney damage amongst young women in Belgium who unsuspectingly took a Chinese herb-based weight loss preparation[74], similar cases have been described in several other countries[3].

6.1. Bioactivation and related toxicities

Available studies suggest that reductive metabolic activation of AAs leads to formation of cyclic reactive nitrenium ion capable of forming covalent DNA and/or protein adducts *(Scheme 6)*[2;5;44]. For example, a study by Stiborova et al.[71] found DNA adducts in the kidney and ureter tissues of Chinese patients who had previously used herbs containing AAs. Furthermore, findings of inhibition and induction studies[65] points to CYP 1A1 or 1A2 as the enzymes likely catalysing bioactivation process of AA in humans. Stiborova et al.[72] have demonstrated the potential of human NQO1 to activate AAI by nitroreduction in the cytosol although they could not rule out possible involvement of cytosolic xanthine oxidase (XO).



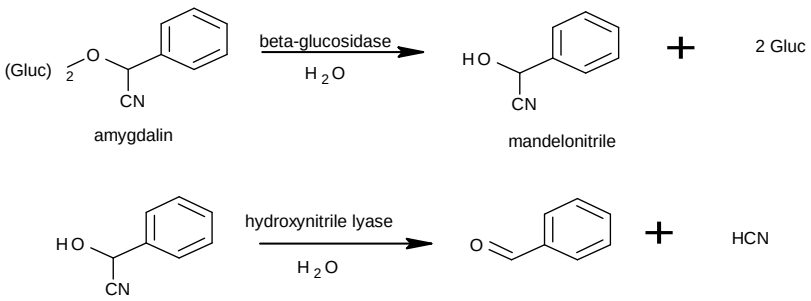
Scheme 6: Metabolic activation of aristolochic acid to cyclic nitrenium ion

7. Cyanogenic Glycosides

Some food plants and seeds are reported to contain cyanogenic glycosides[65]. Cassava for instance is an important source of carbohydrate for people in Africa and South America yet contains the cyanogenic glycosides, linamarin and lotaustralin[17]. Amygdalin is found in seeds of apples and pears, as well as in the leaves, fruit and seeds of black cherry, almond, cherry, plum, peach, and apricot trees at significantly high levels[25]. Other food products such as sorghum and lima beans are known sources of cyanogenic glycosides.

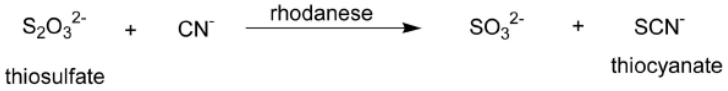
7.1. Bioactivation and related toxicities

The presence of cyanogenic glycosides at a level of 10–50 mg/kg in food is believed to cause goitre which likely occurs via metabolic activation. The β-glucosidases present in the gut flora act on cyanogenic glycosides once ingested to release hydrogen cyanide[17]. For instance, action of β-glucosidase and hydroxynitrilelyase on amygdalin in the GI tract results in HCN *(Scheme 7)*.



Scheme 7: Enzymatic conversion of amygdalin to hydrogen cyanide

A condition known as histotoxic anoxia occurs when cyanide binds to cytochrome oxidase, the terminal enzyme in the mitochondrial electron transport chain. This obstructs ATP generation and oxygen utilization. There are a number of reported case studies with fatal outcome upon ingestion of high levels of amygdalin[31;70].However, acute lethal oral dose of cyanide is reported to vary[1] and sensitivity may be highly dependent on age, body mass and health status of the individual[12]. Although effects of long term exposure to cyanide are less understood, chronic exposure to linamarin from cassavas has been reported to cause malnutrition, diabetes, congenital malformations, neurological disorder, and myelopathy[8;18] One detoxification pathway of cyanide in humans involves conversion in the liver to thiocyanate by the mitochondrial enzyme rhodanese*(Scheme 8).* The less toxic thiocyanate may be excreted in the urine but may also act as iodine antagonist leading to goiter especially in countries with low iodine uptake[55].



Scheme 8: Conversion of cyanide to thiocyanide by rhodanese

Table 1: Overview of selected phytochemicals and their bioactivation pathways leading to toxicity

Compound(s)	Major bioactivation pathway and toxic effects
1. Flavonoids (quercetin)	Formation of quinone-type metabolites as active alkylating DNA-reactive intermediates with genotoxic effects
2 Alkenylbenzenes	Formation of sulfooxymetabolites leading to genotoxic and carcinogenic effects
3. Coumarin	Formation of hepatotoxic intermediate o -hydroxyphenylacetaldehyde via coumarin-3,4-oxide pathway leading to tumour induction
4. Aristolochic acids (AAs)	Formation of reactive nitrenium ion causingnephropathy and urothelial cancers
5. Cyanogenic glycosides	Release of cyanide leading to histotoxic anoxia via cytochrome c oxidase inhibition

8. Interspecies and intraspecies variations

In vivo and in vitro metabolism data obtained from animal studies may not always reflect metabolism in man, due to possible interspecies difference in metabolizing enzymes involved[27]. This may also be the case for phytochemicals. For example, safrole and methyleugenol-induced cancers were more prominent in mice than were seen in rats which suggest possible differences in bioactivation potential and detoxification capacity. Unlike rats, mice were susceptible to 1-hydroxyfrole-2,3-oxide-induced liver cancer. Also, 1-hydroxyestragole-2,3-oxide-mediated lung cancer was only seen in mice. These findings and the overall sensitivity of mice than rats to the alkenylbenzenes possibly reflect the low epoxide hydrolase activity in mice. Furthermore, since the ultimate carcinogen 1-sulfooxymetabolite formation is mediated by CYP and SULF enzymes which are known to exhibit interspecies differences[27], the reliability of extrapolation of these animals data to the human situation without appropriate caveats will be illusory. For instance P450 catalytic activities differ across mammalian species and several SULF enzymes are present in human and absent in rodents[27]. Also, since the proximate carcinogen formation is mediated mainly by CYP 1A1 and 2A6[64], genetic polymorphism is expected to lower the toxicity risk among CYP 2A6 deficient individuals in the human population.

Coumarin toxicity presents yet another example of species difference in bioactivation of phytochemicals. Rodents are more susceptible than humans to coumarin-induced cancer incidence. Whilst bioactivation leading to toxicity was the major pathway in rodents, metabolism in humans favoured detoxification catalyzed by CYP 2A6[36]. However, among human population interindividual variations in coumarin toxicity is expected due to genetic polymorphism in the human CYP 2A6[63]. It cannot be ruled out that the 3,4-epoxidation pathway likely takes over in the metabolism of coumarin in individuals deficient in CYP 2A6 since human CYP 3A13 has the potential to catalyze the 3,4-epoxide route[76]. Polymorphism in aldehyde dehydrogenase is yet another interesting observation which may likely contribute to interindividual differences in sensitivity to coumarin-induced toxicity. Accumulation or delayed clearance of the hepatotoxic intermediate o-HPA is expected in aldehyde dehydrogenase deficient individuals.

Complexities of data extrapolation from animal studies to human situation

The phytochemicals examined in this review are in principle potentially toxic. The fact that these phytotoxins are bioactivated to reactive alkylating intermediates capable of adduction with cellular macromolecules means that they may have associated cellular toxicity and even genotoxicity. However, it must be noted that toxicological outcomes are multifaceted and that factors such as dose, species differences, and interindividual differences in bioactivation as well as toxicodynamic processes may play important roles. Also noteworthy is the fact that whereas animal experiments are in most cases conducted with pure compounds, human exposures often occur in complex matrix. In complex food or herbal-based product matrixes for example, interactions with other ingredients possibly occur which can affect the bioavailability of the phytotoxic components. In addition, other herbal components might interact at the level of metabolic activation and/or detoxification leading to lower adverse effects in such complex matrixes. Another important factor is the time frame between cancer initiation and cancer development. It is difficult, if not impossible, to relate cancer to a specific xenobiotic exposure when the initiating event and actual presence of a tumor may be separated by a span of 20 years.

CONCLUSION

In conclusion,the phytochemicals examined in this review are intrinsically toxic via metabolic activation although overall manifestation is multifaceted and depends on interplay of factors. Importantly, polymorphism in xenobiotic metabolizing enzymes is expected to contribute to interindividual differences in susceptibility to herbal and dietary ingredients. Adduct formation of reactive metabolites with cellular macromolecules appear to mediate adverse effects of phytochemicals although in vivo stability of these adducts play important role in overall manifestation of toxicity. Again, other pathways such as non-genotoxic modes may be relevant in assessment of risk profiles of phytochemicals.The use of herbal and dietary constituents must be done with circumspection because “natural” does not equal “safe” and that adverse effects can occur upon exposure to phytochemicals. The final manifestation however may be delayed making it difficult to trace the cause of ailment.

REFERENCES

1. Agency for Toxic Substances and Disease Registry (ATSDR). Toxicological Profile for Cyanide. U.S. Public Health Service, in collaboration with U.S. Environmental Protection Agency (EPA). 1990. Atlanta, GA, U.S. Public Health Service.
2. Arlt, V. M.; Schmeiser, H. H.; and Pfeifer, G. P. Sequence-specific detection of aristolochic acid-DNA adducts in the human p53 gene by terminal transferase-dependent PCR. Carcinogenesis 2001, 22, 133-140.
3. Arlt, V. M.; Schmeiser, H. H.; and Pfeifer, G. P. Sequence-specific detection of aristolochic acid-DNA adducts in the human p53 gene by terminal transferase-dependent PCR. Carcinogenesis 2001, 22, 133-140.
4. Arlt, V. M.; Schmeiser, H. H.; and Pfeifer, G. P. Sequence-specific detection of aristolochic acid-DNA adducts in the human p53 gene by terminal transferase-dependent PCR. Carcinogenesis 2001, 22, 133-140.
5. Arlt, V. M.; Stiborova, M.; and Schmeiser, H. H. Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. Mutagenesis 2002, 17, 265-277.
6. Arlt, V. M.; Stiborova, M.; and Schmeiser, H. H. Aristolochic acid as a probable human cancer hazard in herbal remedies: a review. Mutagenesis 2002, 17, 265-277.
7. Avila, M. A.; Velasco, J. A.; Cansado, J.; and Notario, V. Quercetin mediates the down-regulation of mutant p53 in the human breast cancer cell line MDA-MB468. Cancer Res. 1994, 54, 2424-2428.
8. Baumeister, R. S. H. Z.-R. G. Toxicological and clinical aspects of cyanide metabolism. Arzneim.Forsch 1975, 25, 1056-1064.
9. Block, G.; Patterson, B.; and Subar, A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. Nutr. Cancer 1992, 18, 1-29.
10. Boberg, E. W.; Miller, E. C.; Miller, J. A.; Poland, A.; and Liem, A. Strong evidence from studies with brachymorphic mice and pentachlorophenol that 1'-sulfooxysafrole is the major ultimate electrophilic and carcinogenic metabolite of 1'-hydroxysafrole in mouse liver. Cancer Res. 1983, 43, 5163-5173.
11. Boersma, M. G.; Vervoort, J.; Szymusiak, H.; Lemanska, K.;

Tyrakowska, B.; Cenas, N.; Segura-Aguilar, J.; and Rietjens, I. M. Regioselectivity and reversibility of the glutathione conjugation of quercetin quinone methide. Chem. Res. Toxicol. 2000, 13, 185-191.
12. Bonsall, J. L. Survival without sequelae following exposure to 500 mg/m3 of hydrogen cyanide. Human Toxicol 1984, 3, 57-60.
13. Born, S. L.; Caudill, D.; Smith, B. J.; and Lehman-McKeeman, L. D. In vitro kinetics of coumarin 3,4-epoxidation: application to species differences in toxicity and carcinogenicity. Toxicol. Sci. 2000, 58, 23-31.
14. Brown, J. P. A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds.Mutat. Res. 1980, 75, 243-277.
15. Burkey, J. L.; Sauer, J. M.; McQueen, C. A.; and Sipes, I. G. Cytotoxicity and genotoxicity of methyleugenol and related congeners-- a mechanism of activation for methyleugenol. Mutat. Res. 2000, 453, 25-33.
16. Burkey, J. L.; Sauer, J. M.; McQueen, C. A.; and Sipes, I. G. Cytotoxicity and genotoxicity of methyleugenol and related congeners-- a mechanism of activation for methyleugenol. Mutat. Res. 2000, 453, 25-33.
17. Cheeke, p. r. Natural toxicants in the feeds, forages, and poisonous plants; interstate publishers: Danville, IL, 1998.
18. Davidson, J. C. Cyanide, cassava, and diabetes. Lancet 1979, 2, 635.
19. Diplock, A. T.; Charleux, J. L.; Crozier-Willi, G.; Kok, F. J.; Rice-Evans, C.; Roberfroid, M.; Stahl, W.; and Vina-Ribes, J. Functional food science and defence against reactive oxidative species. Br. J. Nutr.1998, 80 Suppl 1, S77-112.
20. Dunnick, J. K. and Hailey, J. R. Toxicity and carcinogenicity studies of quercetin, a natural component of foods. Fundam. Appl. Toxicol. 1992, 19, 423-431.
21. EFSA Opinion of the Scientific Panel on Food additives, flavourings, processing aids and materials in contact with food (AFC) on a request from the commission related to coumarin. Question number EFSA-Q-2003-118.104 ed.; 2004; pp 1-36.
22. European Commission. Opinion of the Scientific Committee on Food on Estragole (1-Allyl-4-Methoxybenzene). 2001. Ref Type: Report
23. European Commission. Opinion of the Scientific Committee on Food on Methyleugenol (4-Allyl-1,2-Dimethoxybenzene). 2001. Ref Type: Report
24. European Commission. Opinion of the Scientific Committee on Food on the safety of the presence of safrole (1-allyl-3,4-methylene dioxy benzene) in flavourings and other food ingredients with flavouring properties. 2001. Ref Type: Report
25. European Food Safety Authority Opinion of the Scientific Panel on Food additives, Pfavourings processing Aids and Materials in Contact with Food (AFC) on a request from the commission related to hydrocyanic acid. The EFSA journal 2004, 105, 1-28.
26. Felter, S. P.; Vassallo, J. D.; Carlton, B. D.; and Daston, G. P. A safety assessment of coumarin taking into account species-specificity of toxicokinetics.Food Chem. Toxicol. 2006, 44, 462-475.
27. Guengerich, F. P. comparison of catalytic selective of cytochrome P450 superfamily enzymes from different species. chem. biol. interact. 1997, 106, 161-182.
28. Guenthner, T. M. and Luo, G. Investigation of the role of the 2',3'-epoxidation pathway in the bioactivation and genotoxicity of dietary allylbenzene analogs. Toxicology 2001, 160, 47-58.
29. Guenthner, T. M. and Luo, G. Investigation of the role of the 2',3'-epoxidation pathway in the bioactivation and genotoxicity of dietary allylbenzene analogs. Toxicology 2001, 160, 47-58.
30. Howes, A. J.; Chan, V. S. W.; and Caldwell, J. Structure-Specificity of the Genotoxicity of Some Naturally-Occurring Alkenylbenzenes Determined by the Unscheduled Dna-Synthesis Assay in Rat Hepatocytes. Food and Chemical Toxicology 1990, 28, 537-542.
31. Humbert, i. r.; Tress, i. h.; and braico, k. t. Fatal cyanide poisoning: accidental ingestion of amygdalin. JAMA 1977, 238.
32. Ioannides, c. Bioactivation of chemicals by cytochromes P450. 3[1], 1-9. 2007. environmental

biotechnology. Ref Type: Conference Proceeding
33. Ito, N.; Hagiwara, A.; Tamano, S.; Kagawa, M.; Shibata, M.; Kurata, Y.; and Fukushima, S. Lack of carcinogenicity of quercetin in F344/DuCrj rats. Jpn. J. Cancer Res. 1989, 80, 317-325.
34. Kameoka, S.; Leavitt, P.; Chang, C.; and Kuo, S. M. Expression of antioxidant proteins in human intestinal Caco-2 cells treated with dietary flavonoids. Cancer Lett. 1999, 146, 161-167.
35. Kasahara, Y.; Kiyatake, K.; Tatsumi, K.; Sugito, K.; Kakusaka, I.; Yamagata, S.; Ohmori, S.; Kitada, M.; and Kuriyama, T. Bioactivation of monocrotaline by P-450 3A in rat liver. J. Cardiovasc. Pharmacol. 1997, 30, 124-129.
36. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
37. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
38. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
39. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
40. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
41. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
42. Lake, B. G. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem. Toxicol. 1999, 37, 423-453.
43. Lake, B. G.; Gray, T. J.; Evans, J. G.; Lewis, D. F.; Beamand, J. A.; and Hue, K. L. Studies on the mechanism of coumarin-induced toxicity in rat hepatocytes: comparison with dihydrocoumarin and other coumarin metabolites. Toxicol. Appl. Pharmacol. 1989, 97, 311-323.
44. Lebeau, C.; Arlt, V. M.; Schmeiser, H. H.; Boom, A.; Verroust, P. J.; Devuyst, O.; and Beauwens, R. Aristolochic acid impedes endocytosis and induces DNA adducts in proximal tubule cells. Kidney Int. 2001, 60, 1332-1342.
45. Luo, G. and Guenthner, T. M. Covalent binding to DNA in vitro of 2',3'-oxides derived from allylbenzene analogs. Drug MetabDispos. 1996, 24, 1020-1027.
46. Meerman, J. H.; van Doorn, A. B.; and Mulder, G. J. Inhibition of sulfate conjugation of N-hydroxy-2-acetylaminofluorene in isolated perfused rat liver and in the rat in vivo by pentachlorophenol and low sulfate. Cancer Res. 1980, 40, 3772-3779.
47. middleton,E. and Kandaswami,c. The impact of plant flavonoids on mammalian biology: implications for immunity, inflammation and cancer. In The Flavonoids.J.B. Harborne, ed.; Chapman & Hall, London, 1994; pp 619-652.
48. Miller, E. C.; Swanson, A. B.; Phillips, D. H.; Fletcher, T. L.; Liem, A.; and Miller, J. A. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res. 1983, 43, 1124-1134.
49. Miller, E. C.; Swanson, A. B.; Phillips, D. H.; Fletcher, T. L.; Liem, A.; and Miller, J. A. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res. 1983, 43, 1124-1134.
50. Miller, E. C.; Swanson, A. B.; Phillips, D. H.; Fletcher, T. L.; Liem, A.; and Miller, J. A. Structure-activity studies of the carcinogenicities in the mouse and rat of some naturally occurring and synthetic alkenylbenzene derivatives related to safrole and estragole. Cancer Res. 1983, 43, 1124-1134.
51. miller, N. J. and paganga, g. Structureantioxidant activity relationships of flavonoids and phenolic acids.Free Rad. Biol. Med 1996, 20, 933-956.
52. Mitsunaga, Y.; Kubota, T.; Ishiguro, A.; Yamada, Y.; Sasaki, H.; Chiba, K.; and Iga, T. Frequent occurrence of CYP2D6*10 duplication allele in a Japanese population. Mutat. Res. 2002, 505, 83-85.

53. Morino, K.; Matsukara, N.; Kawachi, T.; Ohgaki, H.; Sugimura, T.; and Hirono, I. Carcinogenicity test of quercetin and rutin in golden hamsters by oral administration. Carcinogenesis 1982, 3, 93-97.
54. National Toxicology Program (NTP). Toxicology and carcinogenesis studies of methyleugenol (CAS No. 93-15-12) in F344/N rats and B6C3F1 mice (gavage studies), Draft NTOTR-491, NIH Publication No. 98-3950. 1998. Ref Type: Report
55. Osuntokun, B. O. Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. World Rev. Nutr. Diet. 1981, 36, 141-173.
56. Pamukcu, A. M.; Yalciner, S.; Hatcher, J. F.; and Bryan, G. T. Quercetin, a rat intestinal and bladder carcinogen present in bracken fern (Pteridiumaquilinum). Cancer Res. 1980, 40, 3468-3472.
57. Phillips, D. H. DNA adducts derived from safrole, estragole and related compounds, and from benzene and its metabolites. IARC Sci. Publ. 1994, 131-140.
58. Qato, M. K. and Guenthner, T. M. 32P-postlabeling analysis of adducts formed between DNA and safrole 2',3'-epoxide: absence of adduct formation in vivo. Toxicol.Lett. 1995, 75, 201-207.
59. Qato, M. K. and Guenthner, T. M. 32P-postlabeling analysis of adducts formed between DNA and safrole 2',3'-epoxide: absence of adduct formation in vivo. Toxicol.Lett. 1995, 75, 201-207.
60. Randerath, K.; Haglund, R. E.; Phillips, D. H.; and Reddy, M. V. 32P-post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 1984, 5, 1613-1622.
61. Randerath, K.; Haglund, R. E.; Phillips, D. H.; and Reddy, M. V. 32P-post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 1984, 5, 1613-1622.
62. Randerath, K.; Haglund, R. E.; Phillips, D. H.; and Reddy, M. V. 32P-post-labelling analysis of DNA adducts formed in the livers of animals treated with safrole, estragole and other naturally-occurring alkenylbenzenes. I. Adult female CD-1 mice. Carcinogenesis 1984, 5, 1613-1622.
63. Rietjens, I. M.; Martena, M. J.; Boersma, M. G.; Spiegelenberg, W.; and Alink, G. M. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol. Nutr. Food Res. 2005, 49, 131-158.
64. Rietjens, I. M.; Martena, M. J.; Boersma, M. G.; Spiegelenberg, W.; and Alink, G. M. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol. Nutr. Food Res. 2005, 49, 131-158.
65. Rietjens, I. M.; Martena, M. J.; Boersma, M. G.; Spiegelenberg, W.; and Alink, G. M. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol. Nutr. Food Res. 2005, 49, 131-158.
66. Rietjens, I. M.; Martena, M. J.; Boersma, M. G.; Spiegelenberg, W.; and Alink, G. M. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol. Nutr. Food Res. 2005, 49, 131-158.
67. Rietjens, I. M.; Martena, M. J.; Boersma, M. G.; Spiegelenberg, W.; and Alink, G. M. Molecular mechanisms of toxicity of important food-borne phytotoxins. Mol. Nutr. Food Res. 2005, 49, 131-158.
68. Schmeiser, H. H.; Pool, B. L.; and Wiessler, M. Identification and mutagenicity of metabolites of aristolochic acid formed by rat liver. Carcinogenesis 1986, 7, 59-63.
69. Scientific Committee on Food (SCF). Opinion on coumarin.1-11. 1999.Brussel , European Commission Health & Consumer Protection Directorate-General. Ref Type: Report
70. Shragg, T. A.; Albertson, T. E.; and Fisher, C. J., Jr. Cyanide poisoning after bitter almond ingestion. West J. Med. 1982, 136, 65-69.
71. Stiborova, M.; Frei, E.; Breuer, A.; Bieler, C. A.; and Schmeiser, H. H. Aristolactam I a metabolite of aristolochic acid I upon activation forms an adduct found in DNA of patients with Chinese herbs nephropathy. Exp. Toxicol. Pathol. 1999, 51, 421-427.
72. Stiborova, M.; Frei, E.; Sopko, B.; Sopkova, K.; Markova, V.; Lankova, M.; Kumstyrova, T.; Wiessler, M.; and

Schmeiser, H. H. Human cytosolic enzymes involved in the metabolic activation of carcinogenic aristolochic acid: evidence for reductive activation by human NAD(P)H:quinoneoxidoreductase. Carcinogenesis 2003, 24, 1695-1703.
73. Stoewsand, G. S.; Anderson, J. L.; Boyd, J. N.; Hrazdina, G.; Babish, J. G.; Walsh, K. M.; and Losco, P. Quercetin: a mutagen, not a carcinogen, in Fischer rats. J. Toxicol. Environ. Health 1984, 14, 105-114.
74. Vanherweghem, J. L.; Depierreux, M.; Tielemans, C.; Abramowicz, D.; Dratwa, M.; Jadoul, M.; Richard, C.; Vandervelde, D.; Verbeelen, D.; Vanhaelen-Fastre, R.; and . Rapidly progressive interstitial renal fibrosis in young women: association with slimming regimen including Chinese herbs. Lancet 1993, 341, 387-391.
75. Vassallo, J. D.; Hicks, S. M.; Daston, G. P.; and Lehman-McKeeman, L. D. Metabolic detoxification determines species differences in coumarin-induced hepatotoxicity. Toxicol. Sci. 2004, 80, 249-257.
76. VovWeimarn, l. b. and Murphy, s. e. CYP 2A13-catalyzed coumarin metabolism: comparison with CYP 2A5 and CYP2A6. Xenobiotica 2003, 33, 78-81.
77. Walle, T.; Vincent, T. S.; and Walle, U. K. Evidence of covalent binding of the dietary flavonoid quercetin to DNA and protein in human intestinal and hepatic cells. Biochem.Pharmacol. 2003, 65, 1603-1610.
78. Wiseman, R. W.; Miller, E. C.; Miller, J. A.; and Liem, A. Structure-Activity Studies of the Hepatocarcinogenicities of Alkenylbenzene Derivatives Related to Estragole and Safrole on Administration to Preweanling Male C57Bl/6J X C3H-Hej F1-Mice. Cancer Research 1987, 47, 2275-2283.