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Botanical Dietary Supplements Gone Bad

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Introduction

The use of botanical dietary supplements has increased exponentially in the past decade. As outlined in the previous reports, they are often perceived to be safe due to their "natural" origin, long-standing ethnomedical use, and over-the-counter availability. However, unlike pharmaceuticals that must undergo extensive clinical trials prior to FDA approval, dietary supplements have little regulation of either efficacy or safety in the USA (1). There is also growing interest in food products with 'functional health' properties, which have only a few regulations worldwide (2). In addition, several of these botanicals are currently extracted using different procedures than they were originally, and over-the-counter availability often leads to over dosing and to severe interactions when taken together with other medications (3-6). In particular, a significant number of these herbal products have been associated with hepatotoxicity (4,7). The ultimate toxin is usually a reactive electrophile or free radical formed by oxidative metabolism. Several examples of botanicals which form reactive intermediates will be discussed in this review.

Kava

Kava (*Piper methysticum*) has been used by the South Pacific Islanders for centuries for spiritual services and as an intoxicating beverage (8). Kava is marketed in North America as a sedative, muscle relaxant, anesthetic, and anticonvulsant (9,10). Kava lactones, such as kawain and methysticin (Figure 1), are considered to be the active constituents of kava extracts responsible for their sedative and anxiolytic effects (9). However, largely due to reports of hepatotoxic effects, several European countries and Canada have banned or severely restricted the sale of kava containing products (11,12). In the USA, the FDA has issued warnings although no restrictions have been placed on the sale of Kava dietary supplements (11).

The mechanism of kava hepatotoxicity is not completely understood; however, it likely involves bioactivation of the methylenedioxy kava lactones to electrophilic *o*-quinones, which can react with glutathione (GSH) and/or covalently modify proteins (11). Incubation of a methanol extract of kava roots with hepatic microsomes in the presence of GSH showed the formation of two GSH conjugates consistent with trapping of the *o*-quinones from methysticin and 7,8-dihydromethysticin (Figure 1) (11). As the corresponding catechols were metabolized extensively to glucuronide and sulfate conjugates, quinoid formation might only occur at high concentrations of kava lactones (11). This and other reports show that kava extracts rich in kava lactones can cause GSH depletion and therefore put undue stress on the liver (13). In addition, it was demonstrated that kava lactones inhibit different proteins, such as various P450 isoforms, the efflux transporter P-glycoprotein (14) and cyclooxygenase enzymes, but there is no direct evidence that the *o*-quinone is responsible for this inhibition (13,15-19).

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Interestingly, it has been reported that the traditional extraction method, which involves maceration of the roots in a water and coconut milk solution, extracts less kava lactones from the rhizomes than observed in commercial products which are usually 96% ethanol or acetone extracts (8,13). This suggests that a reduction of kava lactones in kava products and an optimization of extract preparation could reduce the hepatotoxic potential of kava.

Cascara sagrada

Cascara sagrada was considered “sacred bark” by Native Americans. It consists of the dried bark of *Rhamnus purshianus* (California buckthorn), which is a tree found mainly at the pacific coast of the USA (20). Dietary supplements containing the bark are recommended for the use of acute obstipation for up to 1-2 weeks (21); however, over-the-counter availability makes controlling use difficult. The bark contains various 1,8-dihydroxyanthraquinones as active constituents, mainly derivatives of the aloe-emodin aglycon (Figure 2) (20).

The chronic use of Cascara sagrada involves several risks. Besides cramp like panes in the gastrointestinal tract, chronic use disturbs the electrolyte balance, especially potassium deficiency, causing a circulus vitiosus and a dependency on purgative dietary supplements (20). In addition, several studies reported genotoxic and mutagenic effects *in vitro* and *in vivo* for emodin and its derivatives causing them to be classified as prospective carcinogens (22-28).

The mechanism of toxicity of 1,8-dihydroxyanthraquinones, such as emodin, likely involves redox cycling between the quinone and the semiquinone radical generating reactive oxygen species (ROS) resulting in lipid peroxidation, protein damage, and DNA oxidation (Figure 2) (26,29,30). For example, treatment of Reuber hepatoma and fibroblast Balb/3T3 cells with various anthraquinones resulted in the formation of 8-oxo-dG (26). In addition, concentrations of 50 μM aloe-emodin increased DNA damage as measured by the single-cell gel-electrophoresis assay (COMET assay) (31). Aloe-emodin and other anthraquinones also induced dose-dependently tk-mutations and micronuclei in mouse lymphoma L5178Y cells and inhibited topoisomerase II-mediated decatenation in a DNA decatenation assay (31,32). The authors suggested that anthraquinones bind noncovalently to DNA and inhibit the catalytic function of topoisomerase II, which can lead to DNA breakage, by competing with the DNA binding site of the enzyme (33). It is also possible that anthraquinones can covalently bind to DNA as observed with other quinones, such as *p*-benzoquinone (34,35). Binding of anthraquinones to DNA might also facilitate DNA oxidation due to their high potency of generating ROS. Besides the above mentioned effects of redox cycling by anthraquinones, it is also reported that production of ROS by emodin can cause an immunosuppressive effect in human mononuclear cells and might result in apoptosis in A549 cells (29,36).

More recent long term animal studies regarding the consequences of emodin’s mutagenicity showed no or equivocal evidence for carcinogenic activity (37). In general, the reports suggest that emodin might be a weak genotoxic compound, but it is important to mention that a genotoxic constituent can show genotoxic activities at all dose levels given and can potentially lead to carcinogenic effects. The results in general suggest that Cascara sagrada, might not be genotoxic when consumed under prescribed use conditions; however, chronic use could lead to genotoxic/mutagenic events (21,38). It is important to mention, that Cascara sagrada products are still advertised as slimming or detoxification products; however, considering the potential side effects after chronic use, the intake of this herb for these indications should be restrained. Further in depth studies concerning the potential genotoxic effects of this bark should give more information about their potential adverse activities.

A genotoxic compound is genotoxic at every dose level given

Pennyroyal oil

Pennyroyal oil derived from *Mentha pulegium* or *Hedeoma pulegoides* has been used as an abortifacient and as an insect repellent since Roman times (39). Despite reports of hepatatoxicity, pennyroyal products have been purported to purify blood, alleviate menstrual symptoms, feverish colds, and to act as a digestive (12,40). While no apparent evidence exists for these claimed beneficial activities, there is strong data supporting the hepatotoxic effects of pennyroyal oil (41-43). Small quantities can cause acute liver and lung damage and 10-15 mL of the oil have resulted in death (12). To date, the FDA has not placed restrictions on the internal use of pennyroyal. Therefore, the oil is still available and sometimes advertised as an ailment for menstruation, cold, and various other complaints. The oil primarily contains pulegone plus smaller amounts of several other monoterpenes (menthone, iso-menthone, and neomenthone) that are encountered in mint species (40). *In vivo* metabolism of pulegone is extremely complex, generating dozens of metabolites in urine and bile of treated animals (41). The major bioactivation step involves the oxidation of pulegone by cytochrome P450s to its proximate hepatotoxic metabolite menthofuran (44-47). Subsequently, menthofuran is oxidized to an epoxide which is likely the ultimate hepatotoxic, abortifacient, and carcinogenic compound (Figure 3) (42). It has been shown that the epoxide was capable of covalently binding to GSH and proteins causing hepatic injury (40,42,48,49). Evidence from animal experiments suggests that N-acetylcysteine provides at least partial protection from the hepatotoxic effects of pennyroyal oil (40). Due to these toxic properties of pennyroyal oil, restrictions on the internal use of pennyroyal oil are warranted.

Sassafras oil

The main constituent of Sassafras oil is safrole, which is also present in a number of spices, such as nutmeg, mace, cinnamon, anise, black pepper, and sweet basil (7). Sassafras oil is extracted by steam distillation from the bark and roots of the tree *Sassafras albidum* (50). It has had a traditional and widespread use as a natural diuretic, as well as a remedy against urinary tract disorders or kidney problems until safrole was discovered to be hepatotoxic and weakly carcinogenic (7,51). In 1960 the FDA banned the use of sassafras oil as a food and flavoring additive because of the high content of safrole and its proven carcinogenic effects (52). Several years later the interstate shipment of sassafras bark for making tea was prohibited. However, pure sassafras oil is still available online and also in some health food stores.

Two bioactivation pathways of safrole to potentially hepatotoxic intermediates have been reported (Figure 4) (53,54). The first one involves P450 catalyzed hydroxylation of the benzyl carbon producing 1′-hydroxysafrole and conjugation with sulfate generating a reactive sulfate ester. This ester undergoes a S_N1 displacement reaction creating a highly reactive carbocation which alkylates DNA (Figure 4 A) (53,55). Mice and rats treated with pentachlorophenol, a strong inhibitor of sulfotransferases, showed a decrease in DNA adducts formed by 1′-hydroxysafrole (56,57). The second pathway involves P450 catalyzed hydroxylation of the methylenedioxy ring and formation of the catechol, hydroxychavicol, which is a natural product found in betel leaf (Figure 4 B) (53,58). Hydroxychavicol can easily be oxidized to the *o*-quinone which isomerizes nonenzymatically to the more electrophilic *p*-quinone methide (53). Both pathways could explain the genotoxic effects of safrole and DNA adducts consistent with the carbocation pathway (Figure 4A) have been identified *in vitro* and *in vivo* (59-63). These experiments confirm the genotoxic effects of safrole and thus justify the restrictions made by the FDA and other health authorities.

Aristolochia fangchi

Aristolochic acid (Figure 5), a nitrophenanthrene carboxylic acid, is a natural product found in the plant *Aristolochia fangchi*. The roots of this plant were found in a number of products

sold as “traditional herbal medicines”, as dietary supplements, or weight-loss remedies. Starting in Belgium in the early 1990s, the use of slimming products including Chinese herbs lead to an endemic nephropathy with permanent kidney damage, including end-stage kidney failure requiring dialysis or kidney transplantation (64,65). The same symptoms occurred in patients, who were exposed to botanical preparations containing aristolochic acid, in other European and Asian countries as well as in the United States (66). In addition, aristolochic acid can induce cancer primarily in the urinary tract as observed in some of the patients taking the slimming products (65,67). Further analysis of the dietary supplements revealed that at least in some of these products the roots of *Stephania tetrandra* were inadvertently substituted with the roots of *Aristolochia fangchi*, because of the close similarity of the Chinese names (68). It has been reported that the first step of metabolic bioactivation of aristolochic acid involves reduction of the NO_2 group catalyzed by reductive enzymes such as P450 reductase (Figure 5) (66,69-71). Subsequently, the amino- and carboxyl group cyclize to an aristolactam, which can be oxidized to the N-hydroxylactam by P450 enzymes. The N-hydroxylactam intermediate could be further metabolized by Phase II enzymes, such as sulfotransferases, leading to the formation of reactive esters, which undergo heterolysis of the N-O bond to produce the ultimate carcinogenic and electrophilic nitrenium ion (72). This forms covalent DNA adducts at the exocyclic amino groups of adenine and guanine (64,73). Various DNA adducts were determined with the predominant and most persistent one *in vivo*, 7-(deoxyadenosin-N(6)-yl) aristolactam, which could be found in renal tissues of patients taking aristolochia containing remedies several years after cessation of the product (74). These mutagenic lesions lead to AT - TA transversions*in vitro*, which were identified in high frequency in codon 61 of the c-Ha-ras oncogene in tumors of rodents (75). Based on the structural similarity to methysticin and safrole, another potential bioactivation pathway could involve quinone formation from oxidation of the methylenedioxy ring. Based on all these findings the International Agency of Research on Cancer classified *Aristolochia* species as human carcinogens (76). The European Food Safety Authority (EFSA) raised serious concerns regarding the quality and safety of botanicals containing toxic compounds, such as *Aristolochia* (77). Also, the FDA issued an important alert to health care professionals and dietary trade associations to detain any botanical ingredients that were either labeled as containing the plant *Aristolochia* or may be confused with it and to review their manufacturing procedures to ensure that botanical products are free of aristolochic acids (68).

Conclusions

Although most botanical dietary supplements have health benefits and can be considered safe, as described in previous reports in this form, a few contain compounds, which can be converted to reactive intermediates causing toxicity. There might be various potential toxicities occurring from the intake of botanicals, but the examples shown in this review illustrate the formation of five different reactive intermediates including quinoids, ROS, epoxides, carbocations, and nitrenium ions. Since other botanical dietary supplements contain compounds of similar structure, particularly polyphenols, which can be easily metabolized to reactive electrophiles (34), adverse effects attributed to consumption of dietary supplements could be the result of oxidative metabolism of some constituents to their reactive intermediates. As a result, more research for dietary supplements to evaluate their efficacy and safety is warranted. In addition, regulations of dietary supplements, such as standardization of active or potentially toxic compounds are important as mentioned in other reports of this form.

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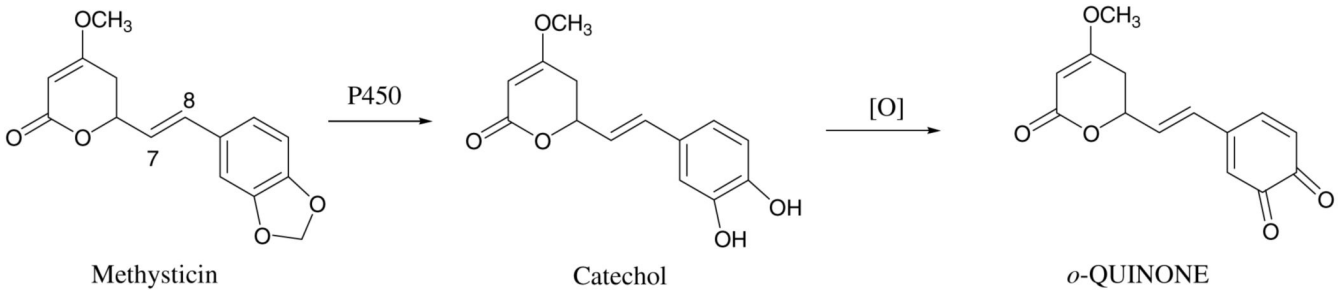

Figure 1.
Bioactivation of methysticin, a kavalactone.

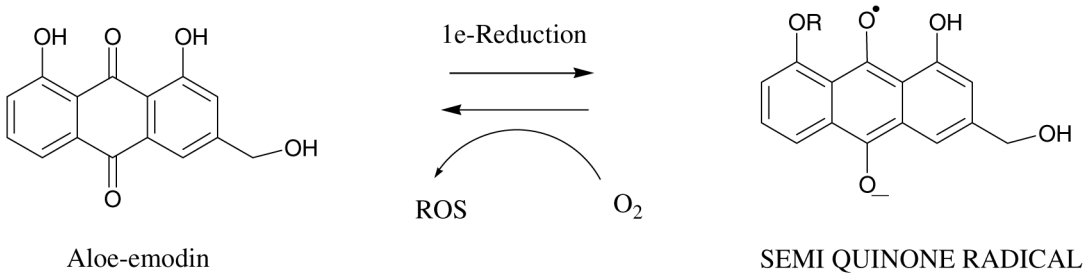

Figure 2.
Redox cycling of Aloe-emodin anthraquinone in Cascara sagrada.

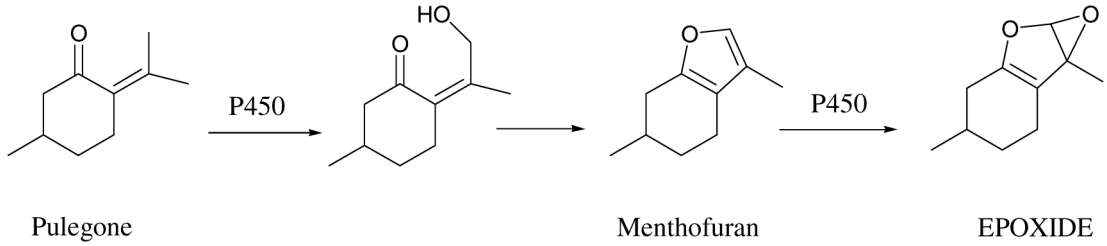

Figure 3.
Bioactivation pathway of pulegone in pennyroyal oil.

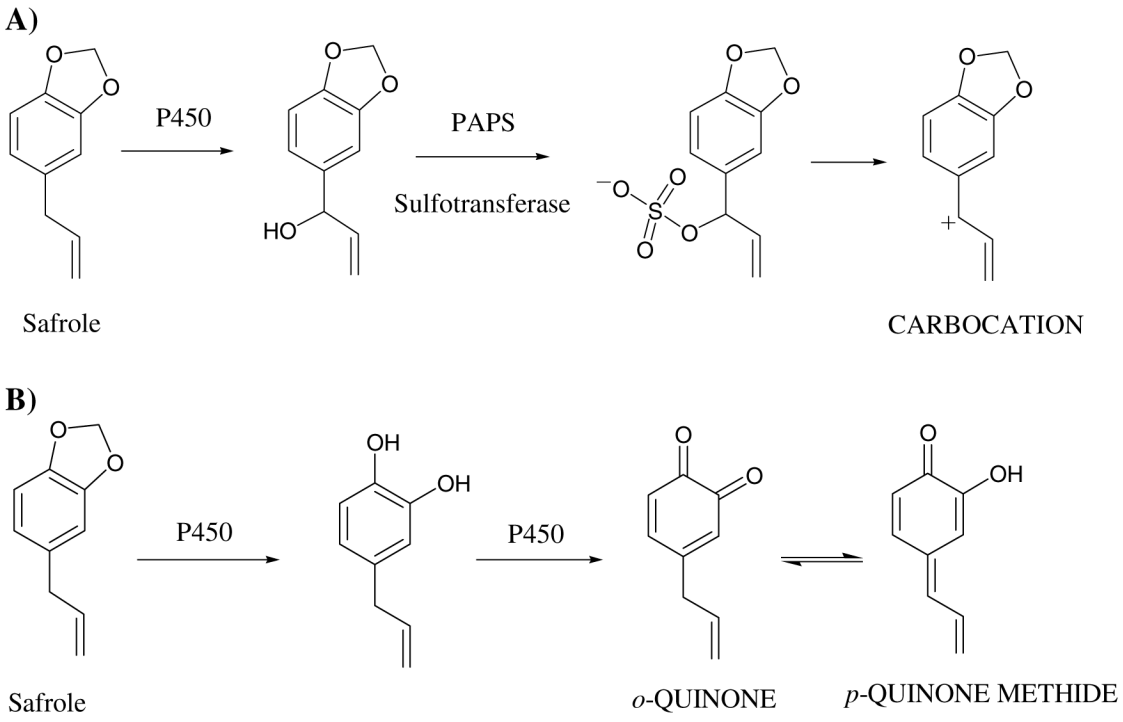

Figure 4.
Two bioactivation pathways of safrole in sassafras oil.

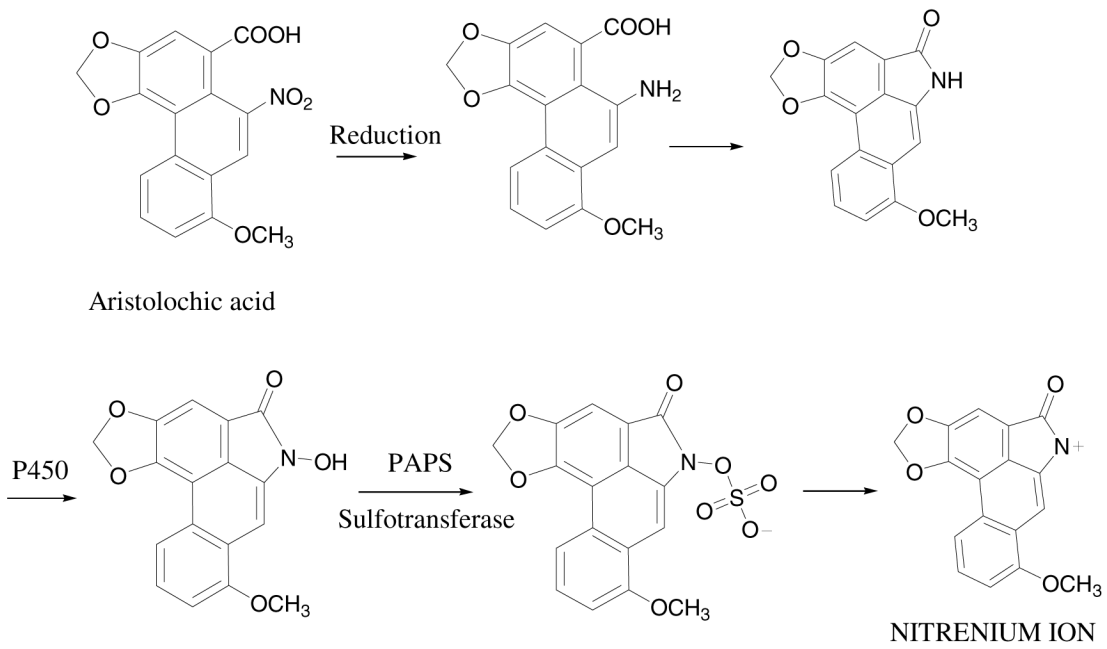

Figure 5.
Bioactivation pathway of aristolochic acid.