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EFFECT OF PYRROLIZIDINE ALKALOIDS FROM TANSY RAGWORT (*SENECIO JACOBAEA*) ON HEPATIC DRUG-METABOLIZING ENZYMES IN MALE RATS

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Abstract—*Ad lib.* consumption of diets containing 5% tansy ragwort (*Senecio jacobaea*) for 1–4 weeks produced a 5- to 6-fold increase in hepatic microsomal epoxide hydrase and significant increases in cytosolic glutathione *S*-transferase activities in male Long–Evans rats. An enhancement of these enzyme activities was also observed when a diet containing 1% tansy ragwort was fed for a period of 3 weeks. Feeding a diet containing 0.5% pyrrolizidine alkaloid (PA) mixture extracted from tansy ragwort for 1 week produced a 5-fold increase in hepatic epoxide hydrase and a 73 per cent increase in glutathione *S*-transferase activities. In contrast, hepatic microsomal aryl hydrocarbon hydroxylase activity (AHH) was reduced significantly by feeding diets containing 5% tansy ragwort or a 0.5% alkaloid mixture. Hepatic microsomal cytochrome P-450 content was lowered following consumption of the 0.5% alkaloid mixture but not by feeding a 5% tansy ragwort diet, the difference presumably being a result of the lowered PA intake by the latter animals. Exposure to the pyrrolizidine alkaloids, therefore, may influence significantly the capacity of animals to metabolize endogenous or foreign compounds and possibly also affect the subsequent biotransformation and toxicity of these plant constituents.

Tansy ragwort (*Senecio jacobaea*), a widely dispersed plant containing hepatotoxic pyrrolizidine alkaloids (PAs), is causing mortalities among grazing animals in the Pacific Northwest and other parts of the world [1]. The plant has invaded pasture lands in the Pacific Northwest, posing an increasing threat to livestock. Humans may also be exposed indirectly, since PAs have been found recently in the milk of tansy-fed cows [2] and in honey produced from the nectar of the tansy ragwort flowers [3].

Mortalities in animals ingesting tansy ragwort are apparently a consequence of irreversible liver damage [1]. In addition to significant pathological changes in the liver and other organs, consumption of tansy ragwort by rats causes a depression in body weight, a modification of organ weights, and altered hematology.§ The toxic constituents of tansy ragwort consist of at least six different PAs: jacobine, jacoline, jaconine, jacozine, senecionine and senecionine [1]. The highest concentration of PAs (approximately 0.2% dry weight) is found in the tansy ragwort flowers [4] with jacobine, the major PA [5], amounting to approximately 60 per cent of the total alkaloids present [4]. Following ingestion, the alkaloids are absorbed from the plant and then undergo conversion in the liver to highly reactive pyrrole metabolites [6–8] capable of alkylating biologically important cellular constituents including proteins and nucleic acids [9–12]. Whether the covalent binding of pyrrole metabolites *in vivo* is solely responsible for the varied toxic effects of PAs is not fully resolved.

Pyrrole metabolites of the PAs are formed primarily in the liver [7, 13] by cytochrome P-450-linked mono-oxygenases requiring O_2 and reduced pyridine nucleotide, and their formation is inhibited by carbon monoxide [8]. Phenobarbital but not 3-methylcholanthrene has been found to enhance pyrrole formation [8].

Little is known about the effects of the PAs on the hepatic drug-metabolizing enzyme system. Shull *et al.* [14] reported previously that a single dose (65 mg/kg) of a PA mixture isolated from tansy ragwort reduced the activity of aminopyrine demethylase and the level of cytochrome P-450 in rat liver. These authors also observed that the *in vitro* microsomal conversion of these alkaloids to pyrroles was reduced in rats pretreated with the same alkaloid mixture. The present study was undertaken to investigate further the effects of the PAs on the activities of hepatic microsomal and cytosolic enzymes in the rat.

MATERIALS AND METHODS

Chemicals. Styrene oxide, styrene glycol and benzo[*a*]pyrene were obtained from the Aldrich Chemical Co. (Milwaukee, WI). [8-^{14}C]Styrene oxide (sp. act. 16.4 mCi/mmole) was purchased from the Amersham/Searle Co. (Arlington Heights, IL). 1-Chloro-2,4-dinitrobenzene (CDNB) was obtained from the Eastman Kodak Co. (Rochester, NY). 3-Hydroxy-benzo[*a*]pyrene was a gift from Dr. D. M. Jerina of the National Institutes of Health, Bethesda, MD.

Animals and diets. Male Long–Evans rats, weighing 150–180 g, were used. One group of animals was fed *ad lib.* a diet that had the following percentage

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composition: ground corn, 58.5; soybean meal, 30; sucrose, 5; corn oil, 3; mineral mix [15], 3; and vitamin mix [16], 0.5. The crude protein content of this diet was 20.8 per cent, dry weight. Two additional groups of animals were fed modifications of this diet, replacing ground corn with 1% or 5% dried, ground tansy ragwort flowers that had been collected in the vicinity of Corvallis, OR. PA intake was estimated on the basis that tansy ragwort flowers typically contain 0.2% alkaloid [4]. Four to six animals from each group were killed at the end of 1, 2, 3 and 4 weeks. Livers were removed, weighed, minced and homogenized for the preparation of cytosol and microsomal fractions.

In another feeding study, a PA mixture extracted from tansy ragwort by the method of Mattocks [17] as modified by Deagen and Deinzer [18] was incorporated into the basal diet at levels of 0, 0.1 and 0.5%. The diets were fed *ad lib.* to male Long–Evans rats (150–180 g) for 1 week, after which the animals were killed and the livers removed and treated as described above.

Preparation of cytosol amd microsomes. Portions (8–10 g) of minced whole livers were homogenized in 1.15% KCl–0.02 M Hepes* buffer, pH 7.4, using a Teflon homogenizer. The homogenate was centrifuged at 10,000 *g* for 20 min. The resulting supernatant fraction was centrifuged at 105,000 *g* for 1 hr using a Spinco model L ultracentrifuge. The 105,000 *g* supernatant (cytosol) fraction was removed and the pellet was resuspended in 1.15% KCl–0.02 M Hepes and respun 105,000 *g* for 45 min. The washed microsomal pellet was then resuspended in KCl–Hepes to the desired protein concentration.

* Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

Analytical procedures. Microsomal and cytosolic protein concentrations were determined according to the method of Lowry *et al.* [19]. Microsomal cytochrome P-450 content was measured by the procedure of Omura and Sato [20] using a Cary 15 recording spectrophotometer. Glutathione *S*-transferase activity was assayed by methods using CDNB and [8-^{14}C]styrene oxide as substrates, essentially as described by Habig *et al.* [21] and James *et al.* [22], respectively. Epoxide hydrase activity was determined using [8-^{14}C]styrene oxide by the thin-layer chromatographic procedure of Jerina *et al.* [23]. Aminopyrine demethylase activity was measured by estimating the amount of formaldehyde formed [24]. Aryl hydrocarbon hydroxylase (AHH) activity was determined by the fluorometric procedure of Nebert and Gelboin [25].

RESULTS

Diets containing 5% tansy ragwort produced a 5- to 6-fold increase in epoxide hydrase activity after a feeding period of 1–4 weeks (Table 1). When 1% tansy ragwort was fed for 3 weeks, epoxide hydrase levels were elevated only 2.3-fold.

With CDNB as substrate, glutathione *S*-transferase activity was significantly increased 24–49 per cent by feeding 5% tansy ragwort at 1, 2, 3 or 4 weeks (Table 1). Increases in glutathione *S*-transferase activity using styrene oxide as substrate were of lower magnitude and were significant only after 1 and 2 weeks of feeding. However, a 24 per cent increase ($P < 0.05$) in glutathione *S*-transferase with the latter substrate was obtained when 1% tansy ragwort was fed for 3 weeks.

Consumption of tansy ragwort did not affect hepatic microsomal cytochrome P-450 levels (Table 2). The activity of hepatic aminopyrine demethylase

Table 1. Effect of feeding tansy ragwort on microsomal epoxide hydrase and cytosolic glutathione *S*-transferase activities in rat liver*

Feeding period (weeks)	Per cent tansy in food	Approximate PA intake ($mg \cdot kg^{-1} \cdot day^{-1}$)	Final body wt (g)	Epoxide hydrase activity [nmoles styrene glycol $\cdot min^{-1} \cdot$ (mg protein)$^{-1}$]	Glutathione *S*-transferase activity [nmoles conjugate $\cdot min^{-1} \cdot$ (mg protein)$^{-1}$)]	
					1-Chloro-2,4-dinitrobenzene	Styrene oxide
1	0	0	228 ± 6	6.20 ± 0.78	1077 ± 66	177 ± 14
	5	8.65	182 ± 5†	30.84 ± 2.27†	1515 ± 68†	227 ± 8‡
2	0	0	264 ± 9	6.93 ± 0.39	998 ± 65	202 ± 12
	5	8.21	199 ± 3†	41.99 ± 3.47†	1488 ± 42†	264 ± 8†
3	0	0	304 ± 14	3.90 ± 0.33	ND‖	239 ± 16
	1	1.90	252 ± 12§	8.97 ± 0.43†	ND	296 ± 13‡
	0	0	325 ± 8	7.75 ± 0.96	1198 ± 71	275 ± 17
	5	8.35	233 ± 5†	39.43 ± 3.60†	1723 ± 113§	316 ± 23
4	0	0	338 ± 18	9.23 ± 0.26	1333 ± 76	357 ± 42
	5	8.04	229 ± 8†	49.96 ± 3.92†	1664 ± 68§	407 ± 27

* Male Long–Evans rats (150–180 g) were fed diets containing 0% (control), 1% or 5% dried and ground tansy flowers and were then killed at various time intervals. Each result is the mean ± S.E.M. of six animals. The probability that the difference between the experimental and control values is statistically significant (determined by Student's *t*-test) is denoted by one of the following symbols: †, ‡, or §.

† $P < 0.001$.

‡ $P < 0.05$.

§ $P < 0.01$.

‖ Not determined.

Table 2. Effect of feeding tansy ragwort on microsomal cytochrome P-450 content and mixed-function oxidase activity in rat liver*

Feeding period	Approximate PA intake ($mg \cdot kg^{-1} \cdot day^{-1}$)	Cytochrome P-450 (nmoles/mg protein)	Enzyme activity ($nmoles\ product \cdot min^{-1} \cdot (mg\ protein)^{-1}$] Aminopyrine *N*-demethylase	Aryl hydrocarbon hydroxylase
1 Week				
Control	0	0.93 ± 0.04	6.84 ± 0.17	0.44 ± 0.02
5% Tansy	8.65	0.77 ± 0.09	5.52 ± 0.35†	0.24 ± 0.06†
2 Weeks				
Control	0	0.84 ± 0.10	7.02 ± 0.27	0.97 ± 0.10
5% Tansy	8.21	0.84 ± 0.13	6.43 ± 0.23	0.54 ± 0.07†

* Male Long–Evans rats (150–180 g) were fed diets containing 0% (control), 1% or 5% tansy ragwort flowers and were then killed at various time intervals. Each result is the mean ± S.E.M. of six animals.
† Indicates a significant difference from control value (determined by Student's *t*-test), ($P < 0.05$).

was slightly but significantly reduced ($P < 0.05$) after 1 week but not after 2 weeks of feeding 5% tansy. The activity of AHH in the liver of tansy-fed rats was reduced approximately 45 per cent ($P < 0.05$) both at 1 and 2 weeks.

Feeding the alkaloid mixture at 0.01 per cent of the diet did not influence the levels of glutathione *S*-transferase in rat liver (Table 3) even though alkaloid intake was comparable to that of the tansy ragwort-fed animals (Table 1). Increasing the level of alkaloid in the diet to 0.5%, however, produced a 5-fold increase ($P < 0.001$) in epoxide hydrase and a 73 per cent increase ($P < 0.01$) in glutathione *S*-transferase activities.

Unlike a diet containing 5% tansy ragwort, consumption of 0.5% alkaloid mixture in the diet caused a small reduction of hepatic microsomal cytochrome P-450 content whereas aminopyrine demethylase was unchanged (Table 4). AHH activity was diminished significantly by both the 0.01% and the 0.5% alkaloid mixture diets (Table 4).

Rats fed the control diets consumed 20–24 g of food/day whereas dietary food intake was reduced to 15–17 g of food/day in the animals fed on 5% ground tansy ragwort. A similar reduction in food intake was observed previously [*, 26]. The rats whose diets contained 0.01% of the PA extract consumed as much food as the controls. The food intake of animals receiving the 0.5% alkaloid diet, however, was quite low (9 g/day) in comparison to the controls (20 g/day). The reduced food intake may have contributed to the relatively lower final body weights of the tansy ragwort- or PA-fed animals (Tables 1 and 3).

The possibility that changes in enzyme activity were due, in part, to decreased food intake was examined in young male Long–Evans rats. One group of animals was allowed to eat only 65 per cent of that consumed daily by the corresponding *ad lib.* fed rats for 1 week. This restriction in food intake did not affect cytochrome P-450 levels or enzyme activities (Tables 5 and 6).

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Table 3. Effect of feeding diets containing a pyrrolizidine alkaloid mixture from tansy ragwort on epoxide hydrase and glutathione *S*-transferase activity in rat liver*

Per cent alkaloid mixture in diet	Approximate PA intake ($mg \cdot kg^{-1} \cdot day^{-1}$)	Final body wt (g)	Enzyme activity [$nmoles\ product \cdot min^{-2} \cdot (mg\ protein)^{-1}$] Epoxide hydrase	Glutathione *S*-transferase
0	0	223 ± 12	3.09 ± 0.33	287 ± 21
0.01	9.67	213 ± 14	3.86 ± 0.22	259 ± 19
0.5	296	150 ± 2†	15.77 ± 1.53†	496 ± 37‡

* Male Long–Evans rats (150–180 g) were fed diets containing 0% (control), 0.01% or 0.5% alkaloid mixture extracted from tansy ragwort and were killed 1 week later. Each result is the mean ± S.E.M. of four animals. [18-^{14}C]Styrene oxide was used as substrate for the two enzyme assays. The probability that the difference (determined by Students *t*-test) is denoted by one of the following symbols: † or ‡.
† $P < 0.001$.
‡ $P < 0.01$.

Table 4. Effect of feeding diets containing a pyrrolizidine alkaloid mixture from tansy ragwort on microsomal cytochrome P-450 content and mixed-function oxidase activity in rat liver*

Per cent alkaloid mixture in diet	Approximate PA intake ($mg \cdot kg^{-1} \cdot day^{-1}$)	Cytochrome P-450 content (nmoles/mg protein)	Enzyme activity [$nmoles\ product \cdot min^{-1} \cdot (mg\ protein)^{-1}$]	
			Aminopyrine *N*-demethylase	Aryl hydrocarbon hydroxylase
0	0	0.76 ± 0.05	6.90 ± 0.34	1.45 ± 0.03
0.01	9.67	0.67 ± 0.06	6.25 ± 0.30	1.20 ± 0.06†
0.5	296	0.61 ± 0.02†	6.97 ± 0.29	0.98 ± 0.09‡

* Male Long–Evans rats (150–180 g) were fed diets containing 0% (control), 0.01% or 0.5% alkaloid mixture extracted from tansy ragwort and were killed 1 week later. Each result is the mean ± S.E.M of four animals. The probability that the difference between the experimental and control values is statistically significant (determined by Student's *t*-test) is denoted by one of the following symbols: † or ‡.

† $P < 0.05$.

‡ $P < 0.01$.

Table 5. Effect of food restriction on hepatic epoxide hydrase and glutathione *S*-transferase activity in male rats*

Type of feeding	Enzyme activity [$nmoles\ product \cdot min^{-1} \cdot (mg\ protein)^{-1}$]	
	Epoxide hydrase	Glutathione *S*-transferase
Ad lib.	4.39 ± 0.27	203 ± 7.7
Restricted	5.02 ± 0.64	196 ± 12.0

* Each value is the mean ± S.E.M. of four animals. The rats on restricted feeding were given feed equivalent to 65% of the amount eaten by the *ad lib.* fed controls. The feeding lasted for 1 week. [8-^{14}C]Styrene oxide was used as substrate for the two enzyme assays.

DISCUSSION

These experiments demonstrate that consumption of tansy ragwort stimulated the activity of hepatic epoxide hydrase and glutathione *S*-transferase in the rat. This response was probably due to the presence of PAs in the plant since feeding an extract alkaloid mixture generally produced a comparable effect. Differences in the response, such as the failure to influence epoxide hydrase and glutathione transferase levels by feeding 0.01% PA in the diet (Table 3) while a comparable PA intake in rats receiving 5% tansy ragwort greatly increased the activity of these enzymes (Table 1), suggests either that the extracted alkaloids are different from the PAs present in tansy ragwort or that there are unknown constituents present in the plant which influence these enzymes. We are presently carrying out further work to determine the effects of the individual pure PAs from tansy ragwort on these enzyme systems.

The increases in epoxide hydrase and glutathione *S*-transferase levels were accompanied by a reduction of AHH activity. This finding is unusual since most previous reports have noted an induction of epoxide hydrase by phenobarbital [23, 27], 3-methylcholanthrene [23, 27] or *trans*-stilbene oxide [28] that paralleled similar increases in microsomal mono-oxygenases [29, 30]. Dietary antioxidants, however, markedly induce epoxide hydrase activity without appreciably influencing cytochrome P-450-dependent systems [31].

The mechanism that produces the differential effects of the PAs on epoxide hydrase, glutathione *S*-transferase and AHH activities is at present not known. The PAs have no appreciable effect on epoxide hydrase, glutathione *S*-transferase and the various mono-oxygenases *in vitro**. PAs are known to inhibit protein synthesis [32, 33], which is consistent with the observed decrease in AHH activity. The increased activities of epoxide and glutathione *S*-transferase in tansy-fed rats may be a selective induction phenomenon or, possibly, a specific acti-

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Table 6. Effect of food restriction on hepatic microsomal cytochrome P-450 content and mixed-function oxidase activity in male rats*

Type of feeding	Cytochrome P-450 content (nmoles/mg protein)	Enzyme activity [$nmoles\ product \cdot min^{-1} \cdot (mg\ protein)^{-1}$]	
		Aminopyrine demethylase	Aryl hydrocarbon hydroxylase
Ad lib.	0.61 ± 0.07	7.08 ± 0.23	1.20 ± 0.08
Restricted	0.77 ± 0.04	7.71 ± 0.04	1.37 ± 0.07

* Each value is the mean ± S.E.M. of four animals. The rats on restricted feeding were given feed equivalent to 65% of the amount eaten by the *ad lib.* fed controls. The feeding lasted for 1 week.

vation of the two enzymes. Metyrapone and 1-(2-isopropylphenyl)-imidazole have been shown previously to be *in vitro* stimulators of epoxide hydrase activity [34] but were inhibitors of the mono-oxygenase systems [35–37]. Since the PAs are known to be carcinogenic in rodents [38], it is noteworthy that Levin *et al.* [39] recently found that epoxide hydrase was significantly induced by various carcinogens; these workers suggested that this enzyme may play some important role in the neoplastic process.

Tansy ragwort PAs such as jacobine are epoxides and could conceivably serve as substrates for epoxide hydrase. In addition, PA pyrroles are capable of forming glutathione conjugates [40], and glutathione *S*-transferase could catalyze this reaction. Alterations in epoxide hydrase and glutathione *S*-transferase levels could, therefore, influence the metabolism and hence the toxicity of the PAs. Comparable effects may also occur in humans consuming food products contaminated with PAs. The role of these enzymes in the toxicity of the PAs, however, remains to be further clarified.

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