Review

Ellagitannins – nature, occurrence and dietary burden

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Abstract: The occurrence of ellagitannins in common foodstuffs is limited to a few fruit and nut species. Dietary intake of ellagitannins is largely explained by the consumption of strawberries, raspberries and blackberries. No reliable figures are available for the ellagitannin burden, but it will probably not exceed 5 mg day^{-1}. Their bioavailability is not well defined. A fraction of the ellagitannins ingested is hydrolysed in the gut and the resulting ellagic acid absorbed and metabolised, but whether intact ellagitannins are absorbed is not clear. There are apparently conflicting claims for beneficial and toxic effects caused by ellagitannins, ellagic acid or ellagitannin-containing extracts in various animal species including rodents and ruminants. It seems unlikely that normal consumption can cause toxic effects in man, but any attempt to increase the intake significantly in pursuit of the suggested benefits should be resisted until the metabolism and pharmacokinetics are better understood.

Keywords: absorption; bioavailability; blackberry; burden; cancer prevention; gallotannins; ellagic acid; ellagitannins; gallagic acid; hydrolysable tannins; metabolism; nuts; pomegranate; raspberry; Rosaceae; strawberry; *Terminalia* spp; yellow-wood; toxicity

INTRODUCTION

Ellagitannins (ETs) are tannins and thereby share common properties with proanthocyanidins: they are water-soluble phenolic compounds of high molecular weight and able to precipitate proteins and alkaloids.[1] They differ from proanthocyanidins in their chemical structures. ETs are esters of hexahydroxydiphenic acid and a polyol, usually glucose or quinic acid (Fig 1).[2] When exposed to acids or bases, ester bonds are hydrolysed and the hexahydroxydiphenic acid spontaneously rearranges into the water-insoluble ellagic acid (EA), hence their name. This reaction forms the basis of an assay commonly used for their detection and quantification.[3] They also differ from proanthocyanidins in their occurrence, which is limited to some dicotyledonous families.

ETs very likely derive from a common gallotannin biosynthetic precursor, penta-*O*-galloyl-β-D-glucose, by the oxidative formation of one or several biphenyl bonds between two or more galloyl residues.[2,4] This galloyl ester intermediate seldom accumulates in plants. In some species, penta-*O*-galloyl-β-D-glucose is further acylated by more galloyl groups to form depsides. Such gallotannins have been extracted from the leaves and galls of *Rhus* spp, from the fruit pods of *Caesalpina spinosa* and from the galls of several oak species. They are known as tannic acid, a generic name for mixtures of gallotannins, and are used for the clarification of beer and fruit juices.

ET monomers can be further oxidised in plants and form dimers, trimers and tetramers with molecular weights up to 4000. The nature of the linkages between monomers, either biphenyl or biarylether, forms the basis of a method of classification.[5,6] Over 150 ET molecules have been isolated from Chinese medicinal plants by Okuda's and Nishioka's groups, who have identified over 150 new structures.[5,7] As components of medicinal plants, they have been traditionally used as astringent drugs to cure diarrhoea, gastric ulcers and burns.[8] They were also shown to exhibit potent antitumour activities in animals when administered ip, iv or po.[9] Much less is known of their biological properties when ingested as part of the human diet. It is the purpose of this review to examine the literature on their occurrence in food, their intake, absorption and metabolism, and to assess their toxicity and potential to produce beneficial effects.

OCCURRENCE IN FOOD

The presence of ETs has been screened in food-yielding plants by the simple detection of EA in

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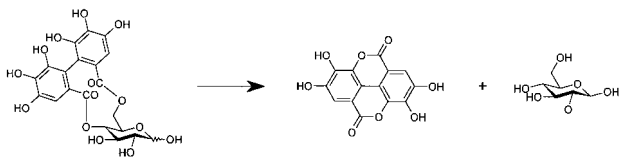


Figure 1. Hydrolysis of ellagitannins.

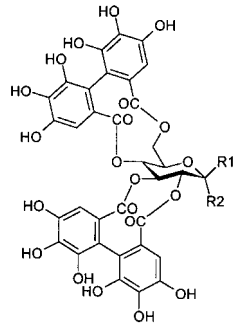
extracts. They were reported to be present in various species of economic importance (Table 1).[2,10] However, such a screening was largely carried out on leaves and not on the edible part of the plant. Very likely, few of these plant species constitute major sources of ETs, either because the tissues containing ETs only represent a small fraction of the edible part (eg the skin of walnut) or because they are not eaten at all. Indeed, ETs often accumulate in a few specific tissues to the exclusion of others.[11] A more detailed investigation on the edible parts of these species is definitely needed.

ETs are most reliably estimated by hydrolysis into EA followed by the estimation of EA by HPLC.[12] When assaying ETs, care should be paid to the selection of the solvent used for hydrolysis, preferentially aqueous alcohol,[13] because the poor solubility of EA in water might otherwise result in an underestimation of ETs.[14] When assaying ETs, free EA must be measured before hydrolysis and subtracted from the value obtained once hydrolysed. EA is naturally present together with ETs in plant tissues or formed during food processing.[15] The proportion of free EA is highly variable and can exceed 50% of the total EA in some samples (compare values before[14] and after[16] hydrolysis of raspberry) depending on the state of the sample (process, storage conditions, etc). It is also important to properly solubilise free EA which may form unrecognised precipitates.[17] This poor solubility explains the apparent drop in EA content upon storage of muscadine grape juice ($7\,mg\,l^{-1}$ in 2 months)[18] (see Ref 19 for more details).

The major contributors to ET intake in Western diets are red fruits such as strawberries, raspberries and blackberries. A survey of the edible parts of 20 different fruit species showed that, apart from these three species, their content of EA, if any, was below the detection limit of the HPLC-UV method ($<0.1\,\mu g\,g^{-1}$ dry weight).[20] In strawberry the total EA content (free + ester forms) estimated after acid hydrolysis accounted for $0.4–0.6\,mg\,g^{-1}$ of the fruit dry weight, with the major part in the pulp and only 4% in the achenes.[20,21] Higher values (1.5 and $8.7\,mg\,g^{-1}$ dry weight in the pulp and achenes respectively) were reported in another study.[22] Tannins were also estimated by precipitation with cinchonine and accounted for 9 mg per fruit ($2\,mg\,g^{-1}$ fresh weight).[23] So far only casuarictin **1** (Fig 2) has been identified in the fruits.[24] Other ETs identified in strawberry leaves are also likely present: for example, the dimer sanguiin H-6 **4** (compound T_1 in Ref 25) and its two constitutive monomers casuarictin **1** and potentillin **2**, and pedunculagin **3** likely formed by hydrolysis of the galloyl residue at C1.[2,25]

Table 1. Ellagitannin-containing plant species of economic importance for food (adapted from Refs 2 and 10)

Family	*Latin name*	*Vernacular name*
Anacardiaceae	*Anacardium occidentale*	Cashew nut
	Pistacio vera	Pistachio
	Mangifera indica	Mango
Betulaceae	*Corylus avellana*	Hazelnut
Ebenaceae	*Diospyros kaki*	Persimmon
Fagaceae	*Castanea sativa*	Chestnut
Juglandaceae	*Juglans regia*	Walnut
Myrtaceae	*Psidium guajava*	Guava
	Eugenia caryophyllata	Cloves
	Pimenta officinalis	Pimento
Punicaceae	*Punica granatum*	Pomegranate
Rosaceae	*Prunus domestica*	Plum
	Prunus armeniaca	Apricot
	Prunus persica	Peach
	Prunus avium	Bird cherry
	Fragaria spp	Strawberry
	Rubus idaeus	Raspberry
	Rubus fruticosus	Blackberry
Saxifragaceae	*Ribes nigrum*	Blackcurrant
	Ribes rubrum	Redcurrant
	Ribes grossularia	Gooseberry
Theaceae	*Camelia sinensis*	Tea
Vitaceae	*Vitis vinifera*	Grape
	Vitis rotundifolia	Muscadine grape



1 R1 = $OCOC_6H_2(OH)_3$; R2 = H
2 R1 = H; R2 = $OCOC_6H_2(OH)_3$
3 R1 = R2 = H, OH

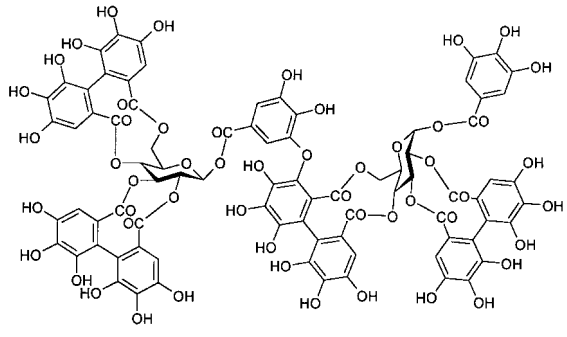


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Figure 2. Structures of compounds **1–4**.

Table 2. Potential dietary sources of ellagitannins consumed in far-Eastern countries

Species	*Edible part*	*Derived products*	*Ellagitannins identified in*	*Reference*
Rosa rugosa var plena	Fruits		Leaves	59
Rosa davurica	Fruits	Vitamin-C health drink	Roots	60
Rubus spp	Fruits		Leaves	59
Trapa japonica	Fruits		Leaves	61, 62
Trapa bicornis				
Psidium guajava	Fruits	Juice, jam	Leaves	63, 64
Psidium cattleianum				
Syzygium jambos	Fruits	Liquor	Leaves	63
Syzygium samarangense				
Feijoa sellowiana	Fruits ('pineapple guava')		Leaves	63
Cornus officinalis	Fruits	Liquor	Fruits	65
Hippophae rhamnoides	Fruits		Leaves	66
Melastoma malabathricum	Fruits, young leaves		Leaves	67
Acer nikoense	Bark and leaves	Health drink	Leaves	68
Acer saccharum	Exudate		Leaves	68
Acer palmatum	Young leaves	In deep-fat-fried food (tempura)	Leaves	68
Geranium thunbergii	Leaves	Herb tea	Leaves	68

In raspberry and blackberry, total EA content reaches 1.2–1.5 and 1.5–2.0 mg g^{-1} dry weight respectively.[20,21] It is largely contained in the seeds (88%).[20] ETs account for the bulk of the total aqueous acetone extracts.[26] In raspberry juice, total EA accounts for 68 mg l^{-1}.[16] Again, sanguiin H-6 **2** is the main ET present in raspberry and blackberry fruits alongside casuarictin **1**, potentillin **2** and pedunculagin **3**.[2,27] The presence of an ET tetramer, lambertianin D, was suggested by comparison of its HPLC retention time with that of a reference sample isolated from another *Rubus* species.[26,28]

Pomegranate juice contains tannins, but no detail was given on their composition.[29,30] Both the fruit peel and the leaves, used as an antidiarrhoeic and anti-helmintic drug and in the treatment of purulent sores, are rich in tannins. Colorimetric assays applied to the pomegranate peel suggested that proanthocyanidins were the major tannins.[31] Different ETs have been identified in the fruit peel,[32–34] bark[35,36] and leaves,[37–39] but it is not known if they are also present in the edible parts.

Mango fruits contain 30–160 mg tannin per fruit, both in the pulp and in the skin.[40] These tannins are probably largely gallotannins similar in structure to sumac and Chinese gallotannins.[41] They have not been characterised in detail. However, β-glucogallin, a monogalloyl ester of glucose, has been isolated and identified in the pulp.[42]

EA was identified in some nuts. It accounted for 0.59 and 0.33 mg g^{-1} dry weight of walnut and pecan nut respectively.[20] One ET, named juglanin, has been isolated from the pellicle of walnut.[43] It is a corilagin isomer, but its structure was not fully established. Sanguiin H-6 **4**, pedunculagin **3** and two other non-identified ET compounds were recognised in the leaves of *Juglans* sp,[25] and several ET monomers, dimers and trimers in those of hazelnut tree, but it is not known whether they are also present in the edible part of the fruits.[44,45] Spices may also contain ETs. The ET eugenin was isolated from cloves and fully characterised.[46]

ETs are also present in some beverages derived from fruits. If hydrolysed, EA is formed and precipitates or induces cloudiness as in wine made out of loganberry (a hybrid between raspberry and blackberry)[47] and in raspberry liqueurs.[48] In muscadine grape juice and wine, EA concentration reaches 20 mg l^{-1}.[49,50] Its content varies according to the fruit processing method[50] and the variety.[51] The content of ETs has not been reported. Several ETs have been identified in the leaves of common grapevine (another *Vitis* sp),[52] but only traces of EA (less than 0.6 mg l^{-1}) could be detected in either green or red grape juice even after hydrolysis.[53] However, EA is a common constituent of wines aged in oak barrels. When aged in a new barrel, its content may reach 50 mg l^{-1}.[54] The EA content in wines depends on the barrel used and on the duration of aging. The leaching of ETs from oak barrels was followed in cognacs over a period of 30 years.[15] Their content reached a maximum of 31 mg l^{-1} after 5 years of aging and then decreased, partly because of hydrolysis into EA. The content of EA consequently increased to 55 mg l^{-1} throughout the aging period.

Beer may also contain some gallotannins which are added as stabilising agents. They form precipitates with proteins and limit their subsequent precipitation with malt proanthocyanidins and the formation of colloidal haze upon storage. Stabilisation is obtained by addition of up to 10 g hl^{-1} tannic acid 24 h prior to the final filtration.[55,56] Residual gallotannins could remain in beer if the quantity added exceeds the capacity of the proteins to bind it.

Tea particularly rich in flavanols also contains some gallotanins and ETs. Two gallotannins (1-*O*-galloyl-β-D-glucose and 1,4,6-tri-*O*-galloyl-β-D-glucose) and an

ellagitannin (1-*O*-galloyl-4,6-(*S*)-hexahydroxydiphenoyl-*β*-D-glucose) have been reported in either fresh tea leaf, commercial oolong or commercial green tea.[57] A much greater range of related compounds has been reported in the leaves of the related species *Camellia oleifera*.[58]

Other edible fruits and leaves consumed in far-Eastern countries could become interesting sources of ETs in Western countries if there was interest in raising ET consumption. However, ETs were often not identified in the edible part, where their occurrence still needs to be confirmed (Table 2).

DIETARY BURDEN

The limited knowledge on the content of ETs in food makes it difficult to evaluate their intake with precision. The main dietary source in France is undoubtedly strawberry. French people consume on average 1.7 kg strawberries as fruit every year (source: CTIFL, Paris) and about as much as transformed products (yoghurts, pastries, syrups, sweets, preserves). This would correspond to a daily consumption of about 0.4 mg total EA. The proportion of EA in the form of ETs is unknown. It is even more difficult to evaluate the contribution of wine consumption (60 l year^{-1} on average in France, mainly as red wine) to ET intake as it depends on its aging in oak barrels. Wine is probably a minor source of ETs as the large majority of the wine consumed is not aged in oak barrels, and most definitely not in ET-rich new barrels.

The daily consumption of EA by Bavarian men and women has been estimated as 4.9 and 5.4 mg day^{-1} respectively.[69] These values, very small in comparison with the total daily polyphenol burden, are probably overestimates, because the values adopted for their contents in wine and grape juice when making the calculation (24 and 15 mg l^{-1} respectively) are unrepresentatively high.

ABSORPTION, METABOLISM AND ELIMINATION

There are no definitive studies on the absorption and metabolism of ETs in man, and their absorption in their native form has never been demonstrated unequivocally even in animals. It has been suggested that gallotannins occurring in commercial tannic acid do cross the intestinal barrier of sheep,[70] but this observation has to be confirmed by experiments using well-characterised pure compounds.

Although it is clear from the results of *in vitro* experiments with intestinal contents and *in vivo* animal studies that hexahydroxydiphenoyl groups in ETs[24] and galloyl groups in gallotannins[71] can be hydrolysed, it is still unclear whether this occurs chemically at the physiological pH of the gut or by the action of the gut microflora.[24] A gallate esterase thought to be of mammalian origin (as distinct from bacterial origin) has been reported in human saliva.[72] There is evidence from studies in which mice were given ETs from raspberries or pomegranates that EA is excreted in the urine (0.05% of the dose) and can be recovered from the lungs at a concentration directly proportional to the dose (60–600 mg kg^{-1} body weight, given by gavage). The EA recovered in lungs reached a maximum of some 6 μg g^{-1} 30 min after dosing, accounted for less than 0.01% of the dose, but recovery was 10-fold higher than from the liver.[73] Virtually no EA was recovered from the blood, lung or liver of mice feeding for 1 week on a diet containing 1% EA,[74] corresponding to a dose of approximately 1 mg kg^{-1} body weight. However, galloyl ester bonds are not necessarily hydrolysed in the orogastrointestinal tract, since intact flavanol gallates have been found in the plasma of volunteers who consumed green tea.[75,76]

There have been studies performed using rats and mice with EA *per se* rather than ETs. Following oral administration of EA to the rat, 10% of the dose was excreted as 3,8-dihydroxy-6*H*-dibenzo[*b,d*]pyran-6-one in urine and faeces.[77] A second metabolite was detected in urine and faeces but not identified. Both metabolites were of microfloral origin. Following intraperitoneal administration, a third metabolite was detected in urine. Small amounts of unchanged EA were detected in faeces of germ-free animals, but none was detected in faeces or urine of any normal rat examined. Urinary metabolites, in contrast to metabolites identified in bile, were essentially non-conjugated.

It is interesting to note that when rats were given EA ip (100 mg kg^{-1} body weight), precipitated EA was found within the abdominal cavity at autopsy,[78] suggesting that its poor water solubility impaired uptake. It may be that the gut microflora can metabolise insoluble EA or that ETs are inherently more soluble, facilitating absorption at least of transformation products.

In contrast to the results obtained with rats, when mice were fed ^{3}H-EA,[79] 28% of the dose (0.12 mg kg^{-1} body weight total EA) was absorbed. EA was largely found intact (80% of all the products identified) in urine, bile and blood, with half of it conjugated with either sulphate, glucuronide or glutathione. Four metabolites were detected but had retention times by HPLC different from those identified by the previous authors. These differences might be explained in part by the difference in the dose applied (about 1000 times lower in the last experiment when expressed per body weight unit), but inter-species differences in metabolism may also exist.

ANTICARCINOGENICITY, ANTITUMORIGENICITY AND TOXICITY

There have been several studies in laboratory animals where either EA or ETs have been given either *per os* or ip, usually in conjunction with a noxious challenge. The challenges have included a range of nitroso compounds (eg *N*-nitrosomethylbenzylamine, diethyl-

nitrosamine, *N*-methylnitrosourea, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, 2,2′-dihydroxy-di-*n*-propylnitrosamine and the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)), 1,2-dimethyl hydrazine, 2-nitropropane, *N*-2-fluorenylacetamide, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, pyrolysis mutagens (PhiP and IQ), PAH, azoxymethane (AOM), 3-methylcholanthrene and chloroform[26,78,80–89] and transplantable tumours (eg MM2 ascites and sarcoma 180).[9]

Ellagic acid

The results of studies using pure EA have been inconsistent. Most studies have reported partial protection against a range of chemical challenges at oral (or ip) doses in the range 4–100 mg kg^{-1} body weight day^{-1} or 0.4–1% of the diet (which for 200 g rats consuming feed equivalent to 10% of their body weight per day would correspond to doses of approximately 300–800 mg kg^{-1} body weight day^{-1}).

In studies of AOM-induced colon cancer[84] and PhiP-induced mammary gland carcinogenesis[83] where EA was given *per os*, no protective effects were seen. In a study where EA was given ip (100 mg kg^{-1} daily) to circumvent problems of solubility and absorption from the gastrointestinal tract, there was apparently partial protection against dietary IQ, but at autopsy, severe liver damage was observed. It was therefore suggested that the partial protection was due to nothing more than decreased metabolic activation of the IQ, being the consequence of non-selective destruction of hepatic cytochromes P-450 by the ellagic acid.[78] This result suggests that the effects of EA may be biphasic.

Ellagitannins

The most comprehensive studies of purified ETs have been those using transplantable tumours, where certain ETs (principally dimers, but also one monomer, five trimers and two tetramers) given ip at 10 mg kg^{-1} have been effective in significantly increasing the lifespan of treated mice relative to controls. To be active, the ET oligomers require a tellimagrandin or potentillin monomer linked C–O–C (as distinct from C–C), in association with an appropriate molecular size and conformation.[9] One such ET is sanguiin H6 as found in raspberries and blackberries. Oral and intravenous dosing were also reported to be effective, but the required doses have not been reported and are presumably considerably greater than those required ip. It has been suggested that the protective mechanism does not involve a direct effect on the tumour cells, but a stimulation of the immune system by activation of macrophages and release of interleukin 1β.

An extract of raspberry ETs containing sanguiin H6 and lambertianin inhibited 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-stimulated DNA synthesis by 42%, TPA-induced ornithine decarboxylase activity by about 30% and TPA-stimulated hydroperoxide production by about 30%.[26] In contrast to raspberry ETs and pure EA, ETs from pomegranate peel containing the anomeric α- and β-punicalagin (10 mg kg^{-1} diet) did not inhibit lung tumorigenesis induced by NNK. Assuming that a 20 g mouse consumed some 2 g feed per day, this diet approximates to a dose of 1 mg kg^{-1} body weight. Punicalagin (2,3-(*S*)-hexahydroxydiphenoyl-4,6-(*S*,*S*)-gallagyl-D-glucose) has been identified as the toxic principle of *Terminalia oblongata* (yellow-wood) responsible for liver necrosis in cattle and sheep and serious economic losses.[90–92] The ip dose of punicalagin required to produce liver lesions in mice has been reported by these workers as 40 mg kg^{-1} body weight in one publication.[92] This is not consistent with a second report[90] where they state it is some 20-fold lower than the oral dose that was reported as 'approximately 0.5–1 mg/kg body wt'. Nor can these results be satisfactorily reconciled with the results of Castonguay *et al*[26] (*vide supra*) who did not report any adverse effect at a 1 mg kg^{-1} (approx) oral dose of the same compound, or with the extensive data of Yoshida *et al*[9] where much larger doses of 81 ETs (but not punicalagin) were given ip without any evidence of harm.

These conflicts cannot be resolved on the current evidence. However, it should be noted that Yoshida *et al*[9] did not report the full autopsy results and apparently did not use a control group receiving only the ET. In such a case it might be that the ET also contributed to the deaths and that the apparent benefit might reflect a situation similar (*vide supra*) to that reported by Ayrton *et al.* [78] It should be noted that the various extracts used in these studies were not necessarily pure. The pomegranate peel extract of Castonguay *et al*[26] contained ill-defined 'oligomeric anthocyanins'. A second toxic principle responsible for vascular renal necrosis without liver necrosis has been identified in yellow-wood, and although described by Oelrichs *et al*[93] as a condensed tannin, this is incorrect, as it is clearly an EA dimer containing four lactone rings, ie the lactonised form of gallagic acid.

A considerable number of other ETs, gallotannins, condensed tannins and galloylshikimic acids have been reported in various *Terminalia* spp, some for the first time.[93–97] Despite containing punicalagin,[95] extracts of bark from *T arjuna* (500 mg, three times per day) have been used for treating coronary patients[98,99] apparently without undesirable side effects. Extracts of *T arjuna* and *T belerica* have also been given to rabbits without ill effects,[100,101] but the composition of the *T belerica* extracts is not known

ANTIMICROBIAL EFFECTS

EA exhibits a dose-dependent inhibitory effect (IC_{50} = 1 mM) on *Helicobacter pylori* isolated from peptic ulcer patients,[102] and ET extracts have been reported to inhibit a range of pathogenic organisms including *Vibrio cholerae, Shigella dysenteriae* and *Campylobacter* spp.[103,104]

HUMAN SIGNIFICANCE

Evidence has been presented for liver damage in rats given EA (100 mg kg^{-1}) ip. In view of the very low absorption, the equipotent oral dose would be much larger and it is difficult to see how this might be encountered in normal human diets. Based on animal studies, the oral doses of EA shown to be partially protective against a range of chemical challenges would correspond to a minimum daily intake of 260 mg EA for a 65 kg adult. The burdens reported by Radtke *et al*[69] are less than one-fiftieth of this figure and indicate that such doses are unlikely in humans, and thus while there would seem to be little risk of harm from dietary EA, there seems even less likelihood of any benefit.

The animal data for ETs are sparse and inconsistent. Punicalagin and gallagic acid dilactone, and plant materials containing them, have all produced toxicity in mice, cattle and sheep. In separate studies, materials containing ETs (some containing punicalagin) have been given to mice, rabbits and humans without obvious ill effect—indeed, some benefits have even been claimed. It is clearly impossible on the present evidence to resolve these conflicting observations or to make recommendations about safety or efficacy in man.

FUTURE RESEARCH REQUIREMENTS

On the evidence currently available, the levels of ETs and EA found in the normal diet are unlikely to be a hazard. However, the information available for the occurrence of ETs in various foods and beverages is inadequate. In view of the evidence for both beneficial effects and hepatotoxicity in rodents and ruminants associated with certain ETs, and the possibility of such ET-rich materials being used therapeutically in man at elevated doses, there is a need to clarify:

- their nature and content in edible products, including processed products;
- the pharmacokinetic properties of both ETs and EA in man;
- their dose–response relationship with particular reference to effects on the liver and kidneys.

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