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PLANT PHENOLS AS IN VITRO INHIBITORS OF GLUTATHIONE S-TRANSFERASE(S)

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Summary: Ellagic acid, a commonly occurring plant phenol, was shown to be a potent in vitro inhibitor of GSH-transferase(s) activity. Other plant phenols such as ferrulic acid, caffeic acid and chlorogenic acid also showed a concentration dependent inhibition of GSH-transferase(s) activity. The I_{50} values of ellagic acid, caffeic acid, chlorogenic acid and ferrulic acid were 8.3 x 10^{-5}M, 14.0 x 10^{-5}M, 20.0 x 10^{-5}M and 22.0 x 10^{-5}M respectively, suggesting that ellagic acid is the most potent inhibitor of all the four studied plant phenols. At 55 μM concentration of ellagic acid, a significant inhibition (35-47%) was observed on GSH-transferase activity towards CDNB, p-nitrobenzyl chloride and 1,2-epoxy-3-(p-nitrophenoxy)propane as substrates. Ellagic acid inhibited GSH-transferase(s) activity in a non-competitive manner with respect to CDNB while with respect to GSH it inhibited the enzyme activity in a competitive manner. Other phenolic compounds purpurogallin, quercetin, alizarin and monolactone also showed a concentration dependent inhibition of the enzyme activity with a I_{50} of 0.8 x 10^{-5}M, 1.0 x 10^{-5}M, 8.0 x 10^{-5}M and 16.0 x 10^{-5}M respectively. These inhibitors of GSH-transferase(s) activity should be useful in studying the in vitro enzyme mediated reactions of exogenous and endogenous compounds.

GSH-transferase(s) (EC 2.5.1.18), a family of cytosolic multifunctional enzymes, play an important role in the biotransformation and detoxification of potential alkylating agents and other xenobiotics (1 - 3). The enzyme catalyses the conjugation of such compounds with the sulphydryl moiety of GSH, thereby neutralizing their active electrophilic sites and subsequently making the parent compund more water soluble (1 - 5). Formation of water soluble glutathione-S-conjugates represents the initial and committing step for further biosynthesis of mercapturic acids (1 - 5). The number of known substrates of GSH-transferase(s) is estimated to be over 3000 (1).

It is well accepted that in order to detoxify the endogenous and exogenous compounds GSH-transferase(s) binds with many organic anions including bilirubin, hormones, dyes and drugs noncovalently, and covalently with carcinogens and/or their metabolites (6 - 8). In addition, there are evidences which suggested that GSH-transferase(s) can also play a role in

Abbreviations used: GSH, glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; GSH-transferase, glutathione S-transferase; PAH, polycyclic aromatic hydrocarbons.

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the activation of certain xenobiotics. For example vicinal dihalogen compounds and 1,2-dibromoethane when reacts with GSH in a reaction catalysed by GSH-transferase(s) forms products which are more mutagenic than the parent compunds (9,10). Futhermore, studies from this laboratory and from others have demonstrated that GSH-transferase(s) can act as a carrier protein for PAH(s) (11,12) which subsequently enhances the microsomal metabolism of bound PAH (11). Besides this, Hammarstrom et al (13) and Parker et al (14) have suggested that GSH-transferase(s) catalyzes the conversion of leukotriene C_4 and thus play an important role in the lipoxygenase pathway of arachidonic acid metabolism.

Considering diversified roles of GSH-transferase(s) the inhibitors and activators of the enzyme will be useful for studies probing the role of the enzyme in various enzyme catalyzed reactions of exogenous and endogenous compounds. During the course of our studies on the role of plant phenols as modulators of PAH metabolism and carcinogenesis (15) we found that certain naturally occurring plant phenols are potent in vitro inhibitors of GSH-transferase(s) activity. These results are discussed in the present communication.

MATERIALS AND METHODS

Rat liver GSH-transferase (a mixture of isoenzymes having activities, 25-50 units per mg protein using CDNB, 2-4 units per mg protein using p-nitrobenzyl chloride and 0.05- 0.50 unit per mg protein using 1.2-epoxy-3-(p-nitrophenoxy) propane as substrates) CDNB, p-nitrobenzyl chloride, 1,2-epoxy-3-(p-nitrophenoxy) propane and ellagic acid were obtained from Sigma Chemical Company, St. Louis, MO. Ferrulic, caffeic, chlorogenic and gallic acids were purchased from Aldrich Chemical Company, Inc., Milwaukee, WI. Quercetin, monolactone, alizarin, purpurogallin and mellitic acid were generously provided by Prof. O. Ratnoff. All other chemicals were of highest purity commercially available.

Enzyme assay:

GSH-transferase activity was assayed with 1-chloro-2,4-dinitrobenzene, p-nitrobenzyl chloride and 1,2-epoxy-3-(p-nitrophenoxy) propane as substrates, as described by Habig et al (16). In order to determine the inhibitor constants (K_i) either CDNB or GSH concentrations were varied at two fixed concentrations of the inhibitor.

RESULTS AND DISCUSSION

The dose dependent inhibitory effects of ferrulic, caffeic, chlorogenic and ellagic acid on GSH-transferase(s) activity using CDNB as a substrate are shown in Fig. 1. Gallic acid which is also a plant phenol, is ineffective in causing a significant inhibition on GSH-transferase(s) activity. Of all the plant phenols, ellagic acid showed maximum inhibition on GSH-transferase(s) activity. The I_{50} values for these plant pheonls for GSH-transferase(s) activity are summarized in Table 1. The lower I_{50} for ellagic acid as compared to other plant phenols further indicates that ellagic acid is more potent in inhibiting GSH-

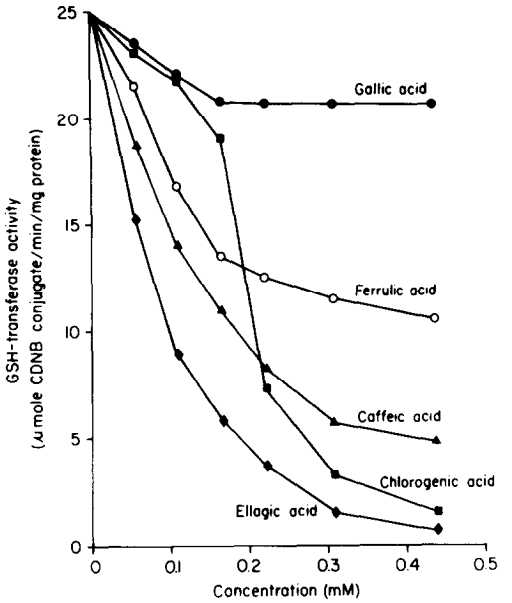
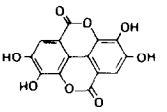
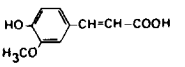
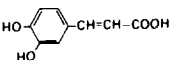
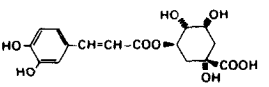


Figure 1: Effect of gallic, ferrulic, caffeic, chlorogenic and ellagic acid on GSH-transferase activity. Data points represent the mean of three replicate determinations.

transferase(s) activity as compared to other plant phenols. The I_{50} for different plant phenols showed the following order: ellagic acid < caffeic acid < chlorogenic acid < ferrulic acid (Table 1).

Table 1: I_{50} values of different plant phenols for GSH-transferase activity using CDNB as a substrate

COMPOUNDS	STRUCTURES	I_{50} for GST
Ellagic acid		8.3×10^{-5} M
Ferrulic acid		22.0×10^{-5} M
Caffeic acid		14.0×10^{-5} M
Chlorogenic acid		20.0×10^{-5} M
Gallic acid		No Inhibition

I_{50} = Concentration of inhibitor which decreased catalytic activity by 50%.

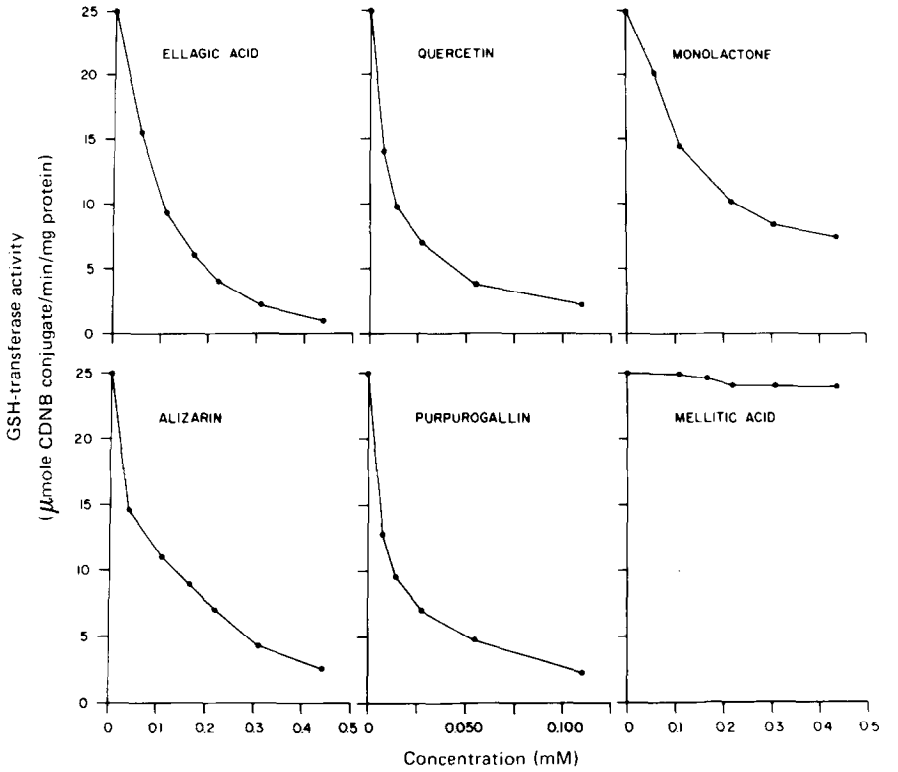
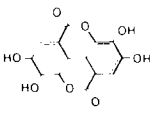
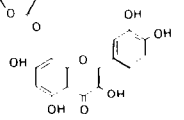
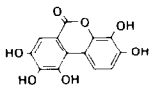
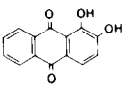
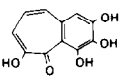
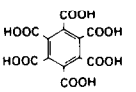


Figure 2: Effect of certain other phenols on GSH-transferase activity. Data points represent the mean of three replicate determinations.

Figure 2 depicts the effect of certain other phenols on GSH-transferase activity using CDNB as substrate. Some of these phenols are also present in plant kingdom (17,18) and have been shown to be carcinogenic and mutagenic (19,20). It is interesting to note that out of five phenolic compounds tested four showed a concentration dependent inhibition of GSH-transferase(s) activity. Mellitic acid is ineffective in inhibiting the enzyme activity. The I_{50} values of these compounds for GSH-transferase(s) activity are given in Table 2 and showed the following order: purpurogallin<quercetin<alizarin<ellagic acid<monolactone.

Figure 3 depicts the effect of ellagic acid on GSH-transferase activity using CDNB, p-nitrobenzyl chloride and 1,2-epoxy-3-(p-nitrophenoxy) propane as substrates. Ellagic acid showed a concentration dependent inhibition of GSH-transferase(s) activity towards all the substrates. The I_{50} of ellagic acid for GSH-transferase(s) activity towards 1,2-epoxy-3-(p-nitrophenoxy)propane, CDNB and p-nitrobenzyl chloride are 6.3×10^{-5}M, 8.0×10^{-5}M and 9.7×10^{-5}M respectively. These results indicate that ellagic acid inhibits GSH-transferase(s) activity towards wide range of substrates. Figure 4 shows the double reciprocal plots with varying concentrations of CDNB and GSH. The V_{max} of GSH-

Table 2: I_{50} values of certain other phenols for GSH-transferase activity using CDNB as a substrate

COMPOUNDS	STRUCTURES	I_{50} for GST
Ellagic acid		8.3×10^{-5}M
Quercetin		1.0×10^{-5}M
Monolactone		16.0×10^{-5}M
Alizarin		8.0×10^{-5}M
Purpurogallin		0.8×10^{-5}M
Mellitic acid		No Inhibition

transferase activity is approximately half in the presence of 55 μM ellagic acid (Fig. 4A). Furthermore, fig 4A also indicates that ellagic acid inhibits GSH-transferase(s) activity in a noncompetitive manner with respect to CDNB with an apparent K_i of 5.5×10^{-5}M. The

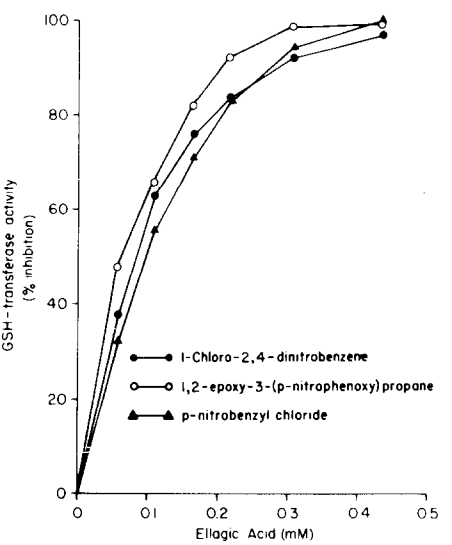


Figure 3: Inhibition of GSH-transferase activity by ellagic acid towards different substrates. Data points represent the mean of three replicate determinations.

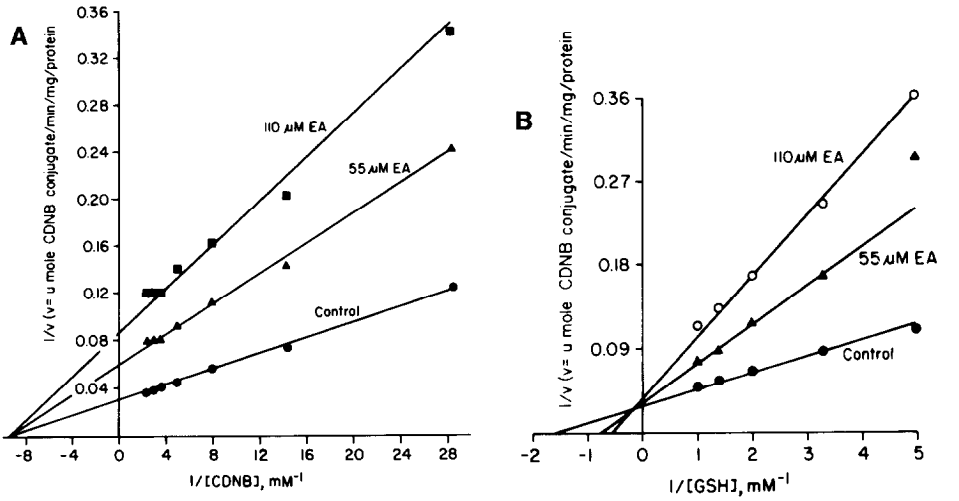


Figure 4: Inhibition constants for GSH-transferase by ellagic acid. K_i was derieved from Lineweaver-Burks plots using varying concentration of CDNB (A) and GSH (B).

V_{max} of GSH-transferase(s) activity with respect to GSH is also found to be decreased with increasing concentrations of ellagic acid (Fig 4B). The inhibition is of competitive manner with respect to GSH with an apparent K_i of 6.7×10^{-3}M (Fig 4B). A similar kind of inhibition on GSH-transferase(s) is observed by indomethacin (21), which is a potent inhibitor of prostaglandin biosynthesis (22). Thus it is quite possible that ellagic acid may also be inhibitory to prostaglandin synthetase pathway which would push the arachidonate molecules to be metabolized by lipoxygenase pathway.

Ellagic acid is a pharmacologically active compund and found to control hemorrhage in humans (23,24) presumably by acting as an activator for instrinsic blood coagulation system (25). This naturally occuring plant phenol at a dose of 50 mg/Kg per day upto 45 days did not show any signs of toxicity (26) and doses of 0.2 mg/Kg intravenously were well tolerated in humans (24). Recent studies of Wood et al (27) have shown that ellagic acid and other plant phenols inhibit the mutagenicity of bay region diol epoxides of PAH in Ames mutagen assay using S. typhimurium. Subsequently, we have shown that ellagic acid inhibits the epidermal metabolism and DNA binding of benzo(a)pyrene (15). Recent studies have shown that ellagic acid offers significant protection against PAH induced tumorigenesis in mice (28,29).

The results of the present investigation suggest that naturally occurring plant phenols are potent in vitro inhibitors of GSH-transferase(s) enzyme. These inhibitors should be

useful in studying the in vitro mechanism of GSH-transferase(s) dependent biotransformation of exogenous and endogenous compunds.

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