

Antioxidant characteristics of phenolic compounds of *Satureia hortensis*

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The antioxidant characteristics of the phenolic water fraction of the annual plant *Satureia hortensis* have been studied. Rabbit liver microsomes served as the oxidative system and arachidonic acid as a substrate. By administering carbon monoxide, NADPH and dimethylaniline the participation of cytochrome P450-dependent monoöxygenase in the oxidation process was examined. Aspirin and phenolic compounds of *Satureia hortensis* were used to study other oxidative systems of rabbit liver microsomes characterized by arachidonic acid consumption. The results obtained showed that arachidonic acid was oxidized in microsomes and the process was intensified in the presence of NADPH. Cytochrome P450-dependent monoöxygenase is not the only oxidizer of arachidonic acid: other oxidative systems of rabbit liver microsomes also take part in this process. The phenolic fraction of *Satureia hortensis* inhibits more strongly than carbon monoxide, a standard inhibitor of cytochrome P450-dependent monoöxygenase and other microsomal oxidative systems. The inhibitory effect manifested *in vitro* correlates with the analogous action of aspirin—one of the primary representatives of nonsteroid anti-inflammatory drugs.

Keywords: arachidonic acid, cytochrome P450 monoöxygenase, inhibition, microsomes, phenolic compounds, *Satureia hortensis*

1. INTRODUCTION

The investigation of biologically active compounds for applications in medicine, the food industry and cosmetics is a topic of high current interest. *Satureia hortensis* is a very interesting test object for such applications since it contains natural phenolic compounds that have previously been found by us to affect some pathological gram-positive and gram-negative microorganisms [1, 2].

The next stage of the work was to study the antioxidant characteristics of phenolic compounds of *Satureia hortensis*. The oxidation of arachidonic (5, 8, 11, 14-eicosatetraenoic) acid (AA) by microsomes of rabbit liver served as the model process. This is a reasonable choice, given the participation of the microsomal enzymatic systems of liver and cytochrome P450-dependent monoöxygenase in the oxidation of AA and other unsaturated fatty acids [3–6]. The inhibitory action of phenolic fractions (F) of *Satureia hortensis* was compared with the analogous actions of carbon monoxide (CO) and aspirin (ASP) in microsomes of rabbits treated with F (*in vivo*) and in microsomes without pretreatment (*in vitro*). The action of phenolic compounds and aspirin on cytochrome

P450-dependent monoöxygenase and cycloöxygenase on AA has been previously well studied [7–10].

2. MATERIALS AND METHODS

2.3–2.5 kg rabbit bucks were selected. Decapitation was followed by liver perfusion with 1.15% KCl solution. Average mass of dried liver was 90–100 g per rabbit. Livers were homogenized and the microsomal fraction isolated [11]. All operations were performed in a cold room at 0–4 °C. Protein was estimated by the Bradford method [12]. The protein concentration was maintained at 2 mg/ml. Cytochrome P450 was determined by the method of Omura and Sato [13].

The oxidative activity of the microsomal fraction was determined by polarography in an open type cell, eliminating the factor of oxygen limitation microsomal suspension was introduced into the polarographic cell.

AA was introduced into the cell as a methanolic solution. The working concentrations were: AA, 33 µM; aspirin, 10 mM; and NADPH, 1.34 µM. 100 µl of the *Satureia hortensis* fraction (see below) was added to a 1% water solution. For inhibition of cytochrome P450-dependent monoöxygenase, the microsomal fraction was saturated with carbon monoxide. The microsomal cytochrome P450 substrate was 100 µl of a saturated solution of dimethylaniline. The phenolic

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fraction (F) of *Satureia hortensis* was prepared using the Zaprometov method [14]; it contains mainly phenolic compounds. *In vivo* F was administered to give a final concentration of 100 mg/kg in per rabbit.

3. RESULTS

In rabbit liver cell microsomes to which the fraction F from *Satureia hortensis* was administered *in vivo*, although the total protein content increased the specific content of cytochrome P450 was reduced by half in comparison with microsomes of intact animals *in vitro* (12.7 ± 1.9 and 23.1 ± 5.7 nM P450 per mg total protein, respectively).

The results of NADPH and dimethylaniline (DMA) oxidation in rabbit liver cell microsomes and the influence of the fraction F and CO *in vitro* and *in vivo* are shown in Fig. 1. These substances appeared to suppress free NADPH oxidation compared with the untreated control (CO by 33%, F by 65%). NADPH-dependent DMA oxidation is suppressed by CO by 23%, by F by 59%, and the combined action of F and CO together inhibits the process by 67%.

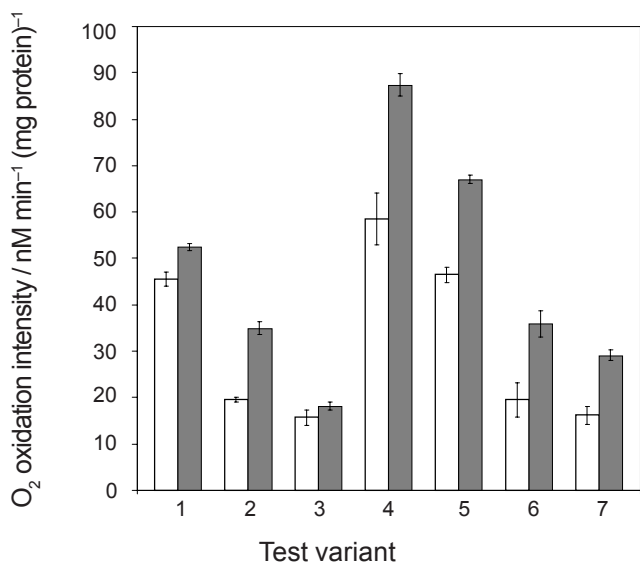


Figure 1. 1=NADPH; 2=NADPH+CO; 3=NADPH+F; 4=DMA+NADPH; 5=DMA+NADPH+CO; 6=DMA+NADPH+F; 7=DMA+NADPH+F+CO; □ *in vivo*, ■ *in vitro*.

On the other hand CO *in vivo* inhibits NADPH oxidation by 57% and F by 66%. CO inhibits DMA oxidation by 21% and F by 67%. The simultaneous action of fraction F and CO inhibits this process by 72%. Our experiments show that F practically affects the entire spectrum of the oxidizing systems of rabbit liver cell microsomes. For cytochrome P450 our results demonstrate higher specific activity of monoöxygenase *in vivo* in comparison with processes *in vitro* (Fig. 2).

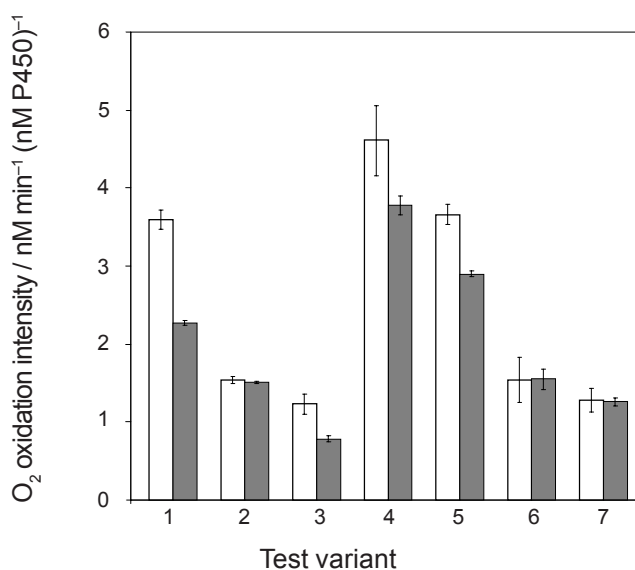


Figure 2. 1=NADPH; 2=NADPH+CO; 3=NADPH+F; 4=DMA+NADPH; 5=DMA+NADPH+CO; 6=DMA+NADPH+F; 7=DMA+NADPH+F+CO; □ *in vivo*, ■ *in vitro*.

Experimental data on the influences of CO, F and aspirin on spontaneous and NADPH-dependent oxidation of AA (Fig. 3) have shown that the functioning of the AA-oxidizing systems *in vivo* is twice as intensive as *in vitro*. The spontaneous oxidation of AA *in vitro* is suppressed by 10% by CO, by 57% by F, and by 62% by aspirin. The same process *in vivo* is inhibited by CO by 53%, by F by 78% and by aspirin by 25%. NADPH more than twice intensifies the oxidizing process in both cases. At the same time CO not only shows an inhibitory effect *in vitro*, but insignificantly strengthens (8%) the NADPH-dependent oxidation of AA. At the same time F inhibits this process by 48%, and aspirin by 80%. On the other hand *in vivo* experiments show that CO inhibits oxidation of AA by 44%, fraction F by 82%, and aspirin by 13%.

Simultaneous administration of CO and F practically totally (97%) suppress as the microsomal activity of AA-oxidizing systems in rabbit liver cells. The specific activity of cytochrome P450-dependent monoöxygenases in this case is higher than for the oxidation of NADPH and DMA (Fig. 4).

4. DISCUSSION

The results of our research have shown that cytochrome P450-dependent monoöxygenase of rabbit liver cell microsomes functions normally (Fig. 1). Note that in our experiments *in vitro* and *in vivo* the presence of cytochrome P420 was not observed. The oxidation of NADPH and DMA in both cases proceeded practically *pari passu*. At the same time the inhibitory action of CO on NADPH oxidation *in vivo*

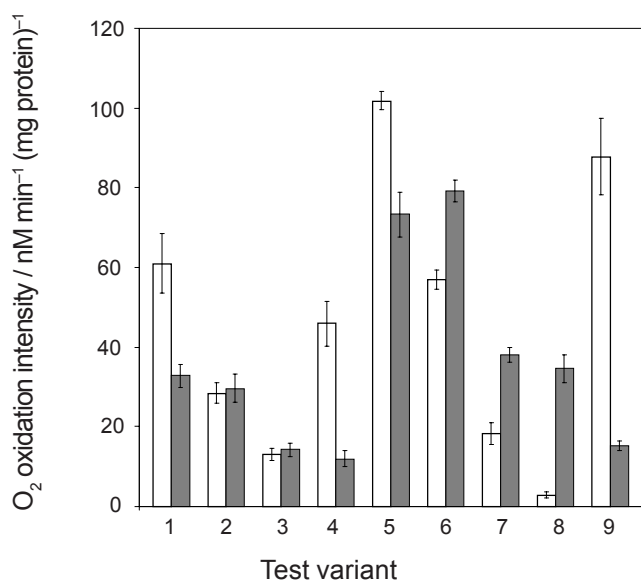


Figure 3. 1 = AA; 2 = AA+CO; 3 = AA+F; 4 = AA+ASP; 5 = AA+NADPH; 6 = AA+CO+NADPH; 7 = AA+NADPH+F; 8 = AA+CO+NADPH+F; 9 = AA+NADPH+ASP; □ *in vivo*, ■ *in vitro*.

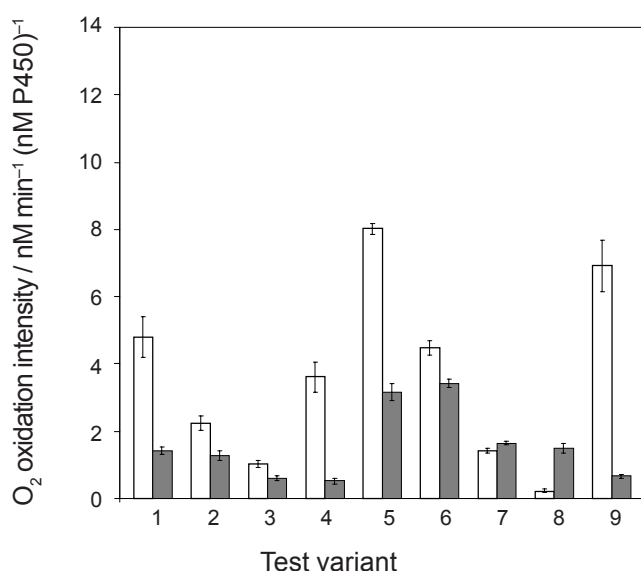


Figure 4. 1 = AA; 2 = AA+CO; 3 = AA+F; 4 = AA+ASP; 5 = AA+NADPH; 6 = AA+CO+NADPH; 7 = AA+NADPH+F; 8 = AA+CO+NADPH+F; 9 = AA+NADPH+ASP; □ *in vivo*, ■ *in vitro*.

is significantly stronger than *in vitro*, whereas F works in both cases more powerfully than CO. These data indicate that the F fraction, unlike CO, is a nonspecific inhibitor and inhibits other NADPH-oxidizing systems apart from monoxygenase. Cytochrome P450 is a membrane-bound enzyme that is influenced by membrane lipid composition [13], while the F fraction is a powerful antioxidant that inhibits lipid peroxidation [15] and in this way promotes the activity of the enzyme. We infer that the preliminary treatment of animals by F strengthens the cytochrome P450-dependent monoxygenase system in rabbit liver cell

microsomes. Though the content of cytochrome P450 in rabbit liver cell microsomes under the influence of fraction F is diminished, the specific activity of cytochrome P450-dependent monoxygenases (Fig. 2) is increased. This effect probably results from certain conformational changes in the structure of the enzymes implicated, raising their efficiency. For the oxidation of DMA in the presence of NADPH the picture is similar.

The study of the processes of AA oxidation, both spontaneous and in the presence of NADPH (Fig. 3), has shown that in both cases the oxidation of AA by rabbit liver cell microsomes proceeds *in vivo* more intensively than *in vitro*. CO inhibits the process *in vitro* by 10%, and *in vivo* by 50%. Aspirin, as expected [9,10], inhibits *in vivo* to a considerably smaller degree. Apparently, preliminary processing of animals with the fraction F in microsomes, in parallel with the activation of monoxygenases, suppresses the cycloxygenase system.

The process of AA oxidation in the presence of NADPH both *in vitro* and *in vivo* proceeds more than twice as intensively in comparison with the spontaneous processes (Fig. 3), presumably because NADPH is the necessary component of many oxidizing systems in microsomes [11].

The stimulating effect of CO on AA oxidation in rabbit liver microsomes that is shown in Fig. 3 could be explained by reference to recent publications [16, 17], according to which some products of P450-dependent oxidation of AA act as an inhibitors of cycloxygenase. In our case preliminary inhibition of P450 by CO blocked P450-dependent AA oxidation and the synthesis of inhibitory products, which increased cycloxygenase oxidative activity (COA). Further application of aspirin inhibited the process of COA. F itself also plays an inhibitory rôle in the process. In animals treated by F (*in vivo*) rather than by aspirin, AA oxidation proceeded differently: the inhibitory effect of CO was more pronounced. This could be explained by proposing that the inhibition of the cycloxygenase pathway by F greatly weakens the inhibitory effect of aspirin. An oppositely inhibitory effect of CO emerged as the process moved towards P450 oxidation. F was a strong inhibitor (both *in vivo* and *in vitro*) of the oxidative enzymatic system, an observation which emphasizes its nonspecific inhibitory properties.

The results obtained have shown that phenolic compounds of *Satureia hortensis* are strong nonspecific inhibitors of monoxygenases and the AA-oxidizing enzymatic systems of rabbit liver cell microsomes. Further investigations will be devoted to

the study of the kinetics and mechanisms of inhibition of AA-oxidation; the composition of the phenolic fractions, and the identification of products of AA-oxidation. The influence of the concentrations of F on these processes *in vivo* will also be investigated.

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