# The Effect of Essential Oil Intake on Changes of Plasma Antioxidant Status in Mice

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**Abstract**

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The aim of this study was to determine the effects of four essential oils intake by feed, namely *Origanum vulgare, Thymus vulgaris, Cinnamomum zeylanicum Ness,* and *Syzygium aromaticum* on antioxidant status in mice *in vivo*. Essential oils were in the aether oleum form. They were diluted with ethanol absolute mixed with ground pelett (0.1, 0.25, 0.57 and 1% concentration) and thereafter ethanol was evaporated. SOD, GPx activities and TAS were measured in erythrocytes and plasma spectrophotometrically with Ransod, Ransel and TAS kits from RANDOX, respectively. GPX activity showed a significant increase in 0.25% and 0.1% concentration of *Origani aetheroleum*. The GPx activities were decreased in 1% concentration of *Thymi aetheroleum* and 0.57% concentration of *Cinnamomi aetheroleum* and 0.57% concentration of *Caryophylli aetheroleum*. The total antioxidant status showed a significant decrease in 1% concentration of *Origani aetheroleum* and significantly increased in 0.1% concentration. The same results were found in *Thymi aetheroleum*. *Cinnamomi aetheroleum* and *Caryophylli aetheroleum* had not effect on total antioxidant status. SOD activities were not significantly changed after intake of essential oils. In conclusion, our results showed, that concentration of essential oil is very important for antioxidant status and also for metabolism of mice, because a high dose of essential oil has adverse effect on metabolism of mice, representated by a lower growth of the body weight. On the other hand, essential oils at lower concentrations have positive effect on antioxidant status of mice.

*Antioxidant activity, vegetable essence, GPx, SOD, TAS*

Antioxidant systems are being shown to play an increasing role in the protection against exogenous oxidative stress. Many authors have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties by spices and essential oils and, in some cases, a direct food-related application has been tested (Madsen and Bertelsen 1995; Sawamura 2000; Horosova et al. 2004; Hsu and Liu 2004a; Masella et al. 2004; Sacchetti et al. 2005). Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases. Exposure to oxidant molecules released from the environment, nutrition or pathologies can generate reactive oxygen species (Faix et al. 2005). Cells have developed enzymatic systems that convert oxidants into non-toxic molecules, thus protecting the organism from the deleterious effects of oxidative stress. SOD is the first line in cell defense against oxidative stress. It converts the superoxide anion $O_2^-$ into a less toxic product, namely $H_2O_2$ and $O_2$ (McCrod et al. 1976). In $H_2O_2$ detoxification, the selenium dependent glutathione peroxidase (GSHPx) converts $H_2O_2$ into water via the oxidation of reduced glutathione (GSH) in oxidized glutathione (GSSG) (Bruce et al. 1982).

The extracts from *Origanum vulgare, Thymus vulgaris, Cinnamomum zeylanicum Ness* and *Syzygium aromaticum* were evaluated for the possible mode of action by studying their antioxidant potential.

## Materials and Methods

During the whole study, the principles of the Ethical committee for the protection of animals in research of the Institute were strictly followed.

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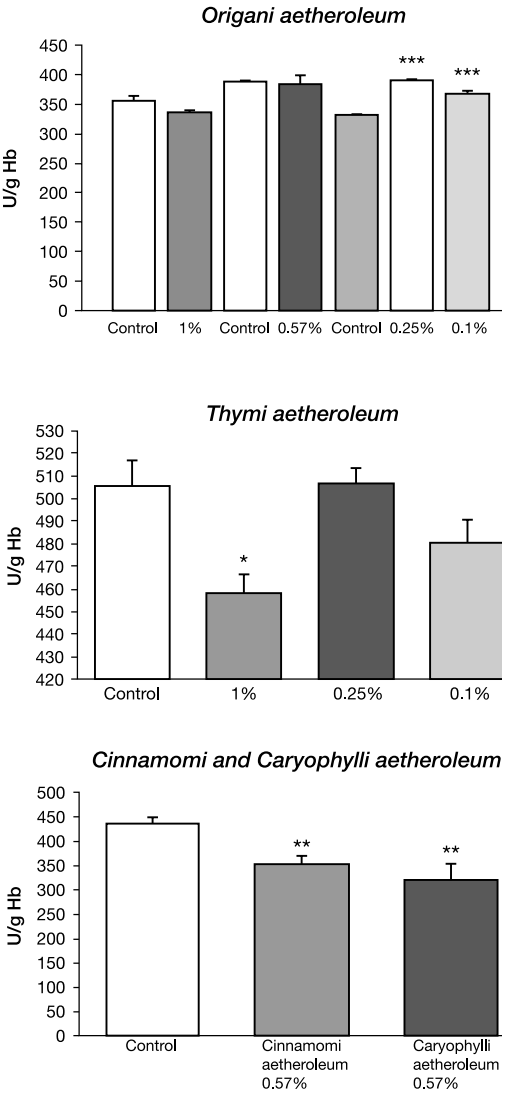


Fig. 1. GPx concentration in blood of mice. Each value represents the mean ± S.E.M.; n = 10 (*$P$ < 0.05, **$P$ < 0.01, ***$P$ < 0.001)

Four essential oils, namely *Origanum vulgare, Thymus vulgaris, Cinnamomum zeylanicum Ness* and *Syzygium aromaticum* were obtained from CALENDULA a.s. (Nová Ľubovňa, Slovak Republic). Ransod, Ransel and TAS kits were obtained from RANDOX Laboratories Ltd, UK.

Male mice (ICR bred), 5-6 weeks of age, were used in the present study. The mice were housed at 22 °C with a 12:12-h dark–light cycle (5.00–17.00 hours lights on). They were maintained on a standard pellet diet for mice with tap water *ad libitum*. Each group contained 10 mice. The control groups were given standard diet for 20 days. Experimental groups were fed standard diet plus supplementation of different essential oils. Essential oils were in aether oleum form. They were diluted with ethanol absolute mixed with ground pellet and thereafter ethanol was evaporated. Final content concentration was different for each essential oil. The final contents of *Origani aetheroleum* were 1%, 0.57%, 0.25% and 0.1%. The final contents of *Thymi aetheroleum* were 1%, 0.25% and 0.1%. *Cinnamomi aetheroleum* and *Caryophylli aetheroleum* were used at 0.57% concentration only. Mice were removed from their cage and rapidly decapitated by guillotine. Blood samples were collected into tubes containing heparin. For SOD determinations, erythrocytes were obtained from 1 ml of blood by centrifugation at 805 *g* for 10 minutes, at room temperature, immediately after the blood was drawn; they were washed three times in a 0.9 mol NaCl solution and stored at -70 °C until analysis. For GPx activity assays, fresh whole blood was collected and stored at -70 °C. Samples were haemolysed by the addition of ice cold distilled water (1/10), cell membranes were removed by centrifugation, and the supernatant was used for the analysis.

SOD (EC 1.15.1.1) and GPx (EC 1.11.1.9) activities were assayed in erythrocytes spectrophotometrically with Ransod and Ransel kits, respectively, using a UV-VIS spectrophotometer. SOD activity was expressed as the amount of protein causing a 50% inhibition of formazan dye (505 nm), employing xanthine and xanthine oxidase to generate superoxide radicals. Units of GPx activity were calculated following NADPH oxidation at 340 nm using cumene hydroperoxide as the substrate. Total antioxidant status was measured in blood plasma by incubation of ABTS with a peroxidase (metmyoglobin) and hydrogen peroxide in the production of the radical cation ABTS• +. This species is blue-green in colour and can be detected at 600 nm.

Data are expressed as mean ± (S.E.M.). The comparison between values was performed by unpaired Student's *t*-test.

## Results and Discussion

The present study evaluated the effects of feeding diets with essential oils contents on oxidative stress in mice. As shown in Fig. 1, GPx activity showed a significant increase in 0.25% and 0.1% concentration of *Origani aetheroleum* (388.7 ± 2.7 and 366.1 ± 4.6 *vs* 329.5 ± 1.6 U·g$^{-1}$ Hb, $P$ < 0.001). The GPX activities were decreased in 1% concentration of *Thymi aetheroleum* (457.7 ± 8.7 *vs* 505.2 ± 11.8 U·g$^{-1}$ Hb, $P$ < 0.05) and 0.57% concentration of *Cinnamomi aetheroleum* (352.0 ± 16.5 *vs* 434.8 ± 13.3 U·g$^{-1}$ Hb, $P$ < 0.01) and 0.57% concentration of *Caryophylli*

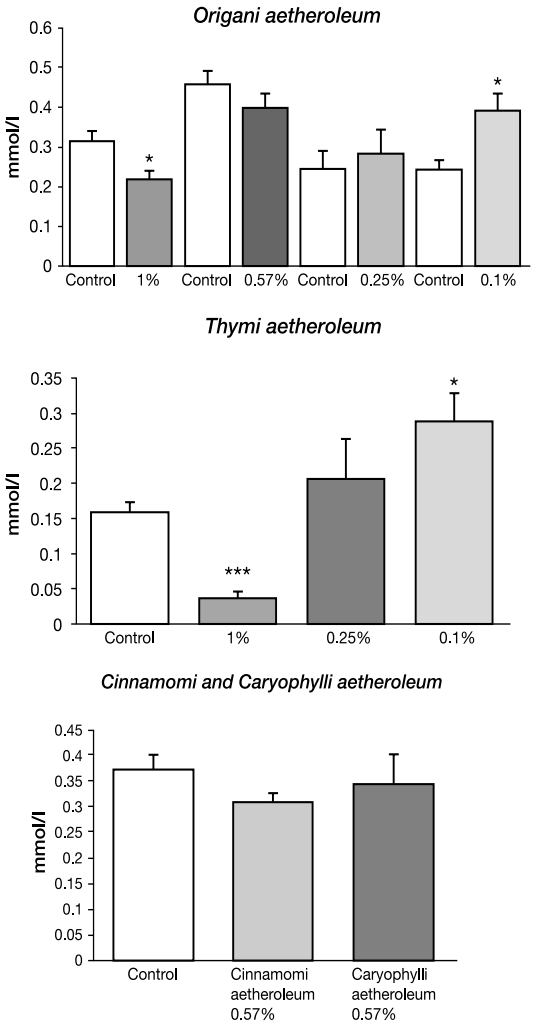


Fig. 2. Total antioxidant status of blood in mice. Each value represents the mean ± S.E.M; n = 10 (*$P < 0.05$, ***$P < 0.001$)

*aetheroleum* (318.1 ± 34.48 vs 434.8 ± 13.28 U·g$^{-1}$ Hb, $P < 0.01$). The reducing activities of GPx at high concentration of essential oils may be a result of pro-oxidative potential. Hodgson and Fridovich (1975) indicated that reduced activities of GPx may result from radical-induced inactivation and glycation of the enzyme.

SOD activities were not significantly changed after essential oils intake. It is possible that the antioxidant properties of essential oils are being utilised by the cells, thus sparing the intracellular antioxidant system. It is also possible that essential oils influenced other cellular systems suggesting that more detailed examination of antioxidant parameters is required.

The protective role of essential oils may result from its antioxidative defense mechanism through the induction of antioxidant enzyme activities. A single dose of sesame oil may attenuate oxidative stress (Hsu and Liu 2004b).

As Fig. 2 shows, total antioxidant status showed a significant decrease in 1% concentration of *Origani aetheroleum* (0.220 ± 0.02 vs 0.315 ± 0.026 mmol·l$^{-1}$, $P < 0.05$) and significant increase in 0.1% concentration (0.389 ± 0.042 vs 0.241 ± 0.026 mmol·l$^{-1}$, $P < 0.05$). The total antioxidant status was decreased in 1% concentration of *Thymi aetheroleum* (0.036 ± 0.009 vs 0.158 ± 0.015 mmol·l$^{-1}$, $P < 0.001$) and increased in 0.1% concentration (0.286 ± 0.04 vs 0.158 ± 0.015 µmol·l$^{-1}$, $P < 0.05$). *Cinnamomi aetheroleum* and *Caryophylli aetheroleum* had no effect on total antioxidant status. Our findings show evidence of an increased sensitivity to oxidative stress at high dose intake of some vegetable extracts. The intake of medicinal plants in rats results in an increase in antioxidant enzyme activity and a decrease in malondialdehyde, which may reduce the risk of inflammation (Choi and Hwang 2005). *Hypericum perforatum* and *Calendula officinalis* hydroalcoholic extracts showed a significant activity (Herold et al. 2003). It will be necessary to find molecules that will increase directly or indirectly the level of antioxidant system. Our results show that essential oils affected oxidative stress in organism and they have positive and negative effect on antioxidant status. In diabetic rats treated with the ethanolic extract, a significant increase in activity of antioxidant enzymes was observed. This might reflect the antioxidant potency of the ethanolic extract, which by reducing blood glucose levels prevented

glycation and inactivation of enzymes (Rajasekaran et al. 2005). Similar kinds of effect, i.e. prevention of potential glycation of antioxidant enzymes and the ensuing decrease in activity, have been reported with other plants, such as *Eugenia jambolana,* well-known for their antidiabetic activity (Ravi et al. 2004).

As Table 1 shows, body weights were significantly lower in 14 day of 1% *Origani aetheroleum* intake (23.41 ± 0.5 vs 27.85 ± 0.4 g, $P < 0.001$) and 7 day of 1% *Thymi aetheroleum* intake (17.4 ± 0.5 vs 21.08 ± 0.4 g, $P < 0.05$). A similar rise in body weight was observed in control and experimental groups of mice with age. Youdim and Deans (1999) show, that increase in body weight of control rats did not differ significantly from that of thyme oil treated rats except at 22 months of age, where control body weights were found to be significantly higher.

A number of previous reports suggest that cellular antioxidants are under homeostatic control and that dietary antioxidant supplementation depresses endogenous antioxidant synthesis so as to nullify the expected beneficial effect of the supplement (Barja de Quiroga et al. 1992; Bunker 1992; Warner 1992).

The oral administration of low doses to mice reduces oxidative stress induced by higher levels of antioxidant enzymes in the plasma. Thus, it appears that these spices exert antioxidant protection through their ability to activate the antioxidant enzymes.

Table 1. Body weight (g) of mice during essential oils intake by diet. Each value represents the mean ± S.E.M.

| | | *Origanum vulgare* | | | |
|---|---|---|---|---|---|
| | Control | 1% | 0.57% | 0.25% | 0.1% |
| 0-day | 20.28 ± 0.20 | 22.21 ± 0.25 | 19.49 ± 0.53 | 21.73 ± 0.86 | 20.83 ± 0.35 |
| 7-day | 25.25 ± 0.31 | 23.70 ± 0.40 | 22.68 ± 0.51 | 25.26 ± 0.31 | 24.94 ± 0.25 |
| 14-day | 27.85 ± 0.43 | 23.41 ± 0.50*** | 25.33 ± 0.77 | 27.99 ± 0.45 | 27.65 ± 0.41 |
| 20-day | 30.41 ± 0.47 | 27.81 ± 1.25 | 27.95 ± 0.85 | 29.58 ± 0.62 | 29.19 ± 0.46 |
| Thymi aetheroleum | | | | | |
| 0-day | 13.05 ± 0.69 | 12.73 ± 0.88 | | 13.18 ± 0.96 | 12.91 ± 0.99 |
| 7-day | 21.08 ± 0.43 | 17.14 ± 0.48* | | 21.34 ± 0.90 | 20.84 ± 1.05 |
| 14-day | 24.16 ± 0.62 | 22.15 ± 0.58 | | 24.24 ± 0.63 | 24.76 ± 0.74 |
| 20-day | 27.01 ± 0.72 | 25.29 ± 0.66 | | 28.40 ± 0.73 | 28.34 ± 0.75 |
| | Cinnamomi aetheroleum | | Caryopilli aetheroleum | | |
| | Control | 0.57% | | 0.57% | |
| 0-day | 13.42 ± 1.28 | 14.93 ± 0.97 | | 13.61 ± 1.01 | |
| 7-day | 20.58 ± 1.26 | 21.37 ± 0.95 | | 19.68 ± 1.07 | |
| 14-day | 25.02 ± 0.92 | 25.84 ± 0.67 | | 25.13 ± 0.69 | |
| 20-day | 27.44 ± 0.79 | 28.89 ± 0.71 | | 28.31 ± 0.49 | |

n = 10. (* $P < 0.05$; *** $P < 0.001$)

## Vplyv éterickych olejov na zmeny antioxidačného statusu v plazme myší

Cieľom práce bolo stanoviť vplyv štyroch éterických olejov *Origanum vulgare, Thymus vulgaris, Cinnamomum zeylanicum Ness* a *Syzygium aromaticum* na antioxidačný status myší *in vivo*. Éterické oleje boli rozpustené v etanole a primiešané do krmiva (pomleté pelety základného krmiva pre myši) v koncentráciách 0,1, 0,25, 0,57 a 1 %. Následne bol etanol z krmiva odparený. V erytrocytoch a plazme sme merali aktivity SOD, GPx a TAS spektrofotometricky Ransod, Ransel a TAS kitmi firmy Randox. GPx bola signifikantne vyššia pri 0,25 a 0,1 % koncentrácii *Origanum vulgare* oproti kontrole. Aktivita GPx sa znížila pri 0,1% koncentrácii *Thymus vulgaris,* 0,57% koncentrácii *Cinnamomum zeylanicum Ness* a 0,57% koncentrácii *Syzygium aromaticum*. Celkový antioxidačný status v plazme bol signifikantne znížený pri 1% koncentrácií a signifikantne zvýšený pri

0,1% koncentrácii *Origanum vulgare.* Podobné výsledky sme zistili pri *Thymus vulgaris, Cinnamomum zeylanicum Ness* a *Syzygium aromaticum*, u ktorých nebol zistený vplyv na celkový antioxidačný status v plazme. Aktivita SOD nebola zmenená po príjme éterických olejov diétou. Záverom, naše výsledky ukazujú, že koncentrácia éterických olejov prijatých krmivom je veľmi dôležitá pre antioxidačný status organizmu a pre metabolizmus myší. Vysoká dávka éterických olejov má nepriaznivý vplyv na metabolizmus myší, predstavujúc znížený nárast telesnej hmotnosti. Na druhej strane, éterické oleje majú v nízkych dávkach pozitívny vplyv na antioxidačný status myší.

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