

Natural phenolic acids may increase serum estradiol level in ovariectomized rats

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Received: 05 June, 2009; revised: 04 August, 2009; accepted: 09 September, 2009
available on-line: 14 September, 2009

Natural phenolic acids are commonly present in plants consumed in the diet. Recently we have observed that different natural phenolic acids exert differential effects on the body mass gain in ovariectomized and non-ovariectomized female rats. The aim of the present study was to investigate the effects of ferulic, caffeic, *p*-coumaric and chlorogenic acids on serum estradiol and total cholesterol levels in ovariectomized and non-ovariectomized rats. The experiments were carried out on 3-month old female Wistar Cmd:(WI)WU rats, divided into following groups (n=8 in each group): non-ovariectomized control rats and non-ovariectomized rats receiving ferulic, caffeic, *p*-coumaric or chlorogenic acids, sham-operated control rats, ovariectomized control rats and ovariectomized rats receiving the same phenolic acids. The phenolic acids were administered at a dose of 10 mg/kg p.o. daily for 4 weeks. Serum estradiol and total cholesterol levels on the next day after the last administration of the phenolic acids were examined. The phenolic acids did not affect serum estradiol or total cholesterol levels in non-ovariectomized rats. In ovariectomized rats, caffeic acid and to a lesser extent *p*-coumaric acid increased serum estradiol level, which effect correlated with a decreased body mass gain. All the phenolic acids decreased serum cholesterol level in ovariectomized rats. Concluding, the anti-obesity activity of some phenolic acids may be, at least partially, connected with estrogenic pathways.

Keywords: ferulic acid, caffeic acid, *p*-coumaric acid, chlorogenic acid, estradiol, body mass, rat

INTRODUCTION

Phenolic acids are secondary metabolites of vascular plants. Secondary metabolites are involved in numerous aspects of the plant life, ranging from structural to protective ones (Stalikas, 2007).

In animals, phenolic acids have been reported to exert antiinflammatory, antioxidant, antimutagenic, anticarcinogenic, and body mass reducing activities (Stalikas, 2007; Hsu & Yen, 2008). In our previous reports we have observed that different natural phenolic (hydroxycinnamic) acids exert differential effects on the body mass gain in female rats, depending on the rat estrogen status (Folwarczna *et al.*, 2009; Zych *et al.*, submitted). Administration of the phenolic acids at a dose of 10 mg/kg p.o. dai-

ly for 4 weeks to ovariectomized or non-ovariectomized rats slowed their body mass gain. However, the effects of caffeic and *p*-coumaric acids were more pronounced than those of ferulic and chlorogenic acids in the ovariectomized rats (Folwarczna *et al.*, 2009). In contrast, chlorogenic and to a lesser extent ferulic acids were more effective than caffeic and *p*-coumaric acids in non-ovariectomized rats (Zych *et al.*, submitted). These observations indicate the possibility of their interfering with estrogen receptors or downstream pathways.

The aim of the present study was to investigate the effects of ferulic, caffeic, *p*-coumaric and chlorogenic acids on serum estradiol, total cholesterol and triglyceride levels in bilaterally ovariectomized and non-ovariectomized rats.

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METHODS

The experiments were carried out on 3-month-old female Wistar Cmd:(WI)WU rats, fed a standard diet *ad libitum*. The rats were obtained from the Medical Research Center, Polish Academy of Sciences (Warszawa, Poland). The procedure of the experiments on animals was approved by the Local Ethics Commission (Katowice, Poland).

The investigated phenolic acids: ferulic (purity 99%), caffeic (purity $\geq 98\%$), *p*-coumaric (purity $\geq 98\%$) and chlorogenic (purity $\geq 95\%$) were purchased from Sigma-Aldrich. The phenolic acids were administered, by a stomach tube (p.o.), at a dose of 10 mg/kg, once daily for 4 weeks. Control rats received the vehicle – distilled water at the same volume of 2 ml/kg.

The experiments were carried out on non-ovariectomized and ovariectomized rats. The non-ovariectomized animals were divided, according to the received treatment, into 5 groups: I. Control rats, II. Ferulic acid, III. Caffeic acid, IV. *p*-Coumaric acid, V. Chlorogenic acid. In the experiment on ovariectomized rats, the animals were divided into 6 groups: I. Sham-operated control rats, II. Ovariectomized control rats, III. Ovariectomized rats receiving ferulic acid, IV. Ovariectomized rats receiving caffeic acid, V. Ovariectomized rats receiving *p*-coumaric acid, VI. Ovariectomized rats receiving chlorogenic acid. Bilateral ovariectomy was performed 7 days before the start of administration of the phenolic acids or vehicle. Each group consisted of 8 animals.

The body mass of rats at the start of administration of the phenolic acids or vehicle was 190–220 g for non-ovariectomized rats and 200–240 g for ovariectomized rats. During the experiment, the rats were weighed 3 times a week.

The next day after the last administration of the phenolic acids, blood samples were collected by heart puncture (under ether anaesthesia) into tubes without an anticoagulant. The animals were fasted overnight before the blood collection. After the samples had stood at room temperature for 2 h, serum was prepared by centrifugation at $1500\times g$ for 10 min. The serum was stored below -20°C until analyzed. Mass of the uterus and thymus, which are estrogen-sensitive organs, isolated from sacrificed animals, was also determined.

Total cholesterol and triglyceride concentrations in the serum were determined colorimetrically, using kits produced by Pointe Scientific, Inc.

Serum estradiol concentrations were determined by competitive ELISA using a commercially available kit produced by DRG Instruments GmbH. Prior to the ELISA procedures, rat serum was extracted with diethyl ether, following the instructions

provided by the producer (based on the method of Hany *et al.*, 1999).

Statistical analysis. Results are presented as the mean \pm S.E.M. One-way ANOVA followed by *post-hoc* Duncan's test was used for evaluation of statistical significance of the results. The results obtained after administration of the phenolic acids to non-ovariectomized or ovariectomized rats were compared with those of appropriate control rats.

RESULTS

Effects of phenolic acids on body mass gain

After a 4-week period of administration of the phenolic acids (10 mg/kg p.o. daily) to non-ovariectomized rats, their body mass gain tended to decrease (not shown). The effects of chlorogenic (mean decrease by 20.9%) and ferulic acid (21.5%) were stronger than those of caffeic and *p*-coumaric acids (8.9 and 16.0%, respectively).

During 4 weeks of observation, the ovariectomized control rats achieved a much higher body mass gain than the sham-operated control rats (by 139%), which is a well-established effect of estrogen deficiency (Table 1). Caffeic and *p*-coumaric acids statistically significantly decreased the body mass gain in estrogen-deficient rats. Ferulic and chlorogenic acids exerted weaker, non-significant, effects.

Effects of phenolic acids on uterus and thymus mass

Administration of the phenolic acids did not affect the mass of the uterus or thymus in non-ovariectomized rats (not shown).

Bilateral ovariectomy induced a substantial decrease of the uterus mass and an increase of the thymus mass in relation to the sham-operated controls, expected results of a lowered estrogen level. Although administration of the phenolic acids did not affect uterus mass, caffeic, *p*-coumaric and chlorogenic acids markedly decreased the mass of the thymus in the ovariectomized rats (Table 1).

Effects of phenolic acids on serum levels of estradiol, total cholesterol and triglycerides

The phenolic acids did not affect the serum levels of total cholesterol, triglycerides or estradiol in non-ovariectomized rats (not shown).

In the ovariectomized control rats, the level of estradiol was statistically significantly decreased in comparison with the sham-operated controls (Fig. 1). Administration of caffeic acid statistically significant-

Table 1. Effects of natural phenolic acids on uterus and thymus mass and on body mass gain in ovariectomized (OVX) rats

Parameter	Sham-operated control	OVX control	OVX + ferulic acid	OVX + caffeic acid	OVX + <i>p</i> -coumaric acid	OVX + chlorogenic acid
Body mass gain after 4 weeks [g]	20.6±2.3	49.2±2.3***	43.3±2.3***	39.5±1.7*** °	40.7±1.7*** °	43.9±4.2***
Uterus mass [g]	0.663±0.128	0.099±0.007***	0.088±0.006***	0.088±0.008***	0.103±0.004***	0.094±0.003***
Thymus mass [g]	0.352±0.017	0.746±0.032***	0.711±0.029***	0.613±0.023*** °°	0.598±0.033*** °°	0.618±0.030*** °°

The phenolic acids (10 mg/kg p.o. daily) were administered for 4 weeks. Results are presented as means ± S.E.M. *** $P < 0.001$, statistically significantly different from the sham-operated control group. ° $P < 0.05$, °° $P < 0.01$, statistically significantly different from the OVX control group.

Table 2. Effects of natural phenolic acids on levels of cholesterol and triglycerides in ovariectomized (OVX) rats.

Parameter	Sham-operated control	OVX control	OVX + ferulic acid	OVX + caffeic acid	OVX + <i>p</i> -coumaric acid	OVX + chlorogenic acid
Total cholesterol [mg/dl]	59.9±2.9	94.7±4.2***	83.5±3.3***	79.3±4.3** °	79.7±2.9** °	81.3±4.9*** °
Triglycerides [mg/dl]	56.7±13.9	62.7±11.6	57.9±5.3	53.6±6.4	54.2±4.5	62.9±7.2

The phenolic acids (10 mg/kg p.o. daily) were administered for 4 weeks. Results are presented as means ± S.E.M. ** $P < 0.01$, *** $P < 0.001$, statistically significantly different from the sham-operated control group. ° $P < 0.05$, statistically significantly different from the OVX control group.

ly increased and administration of *p*-coumaric acid tended to increase the serum estradiol level.

Total cholesterol level in the serum of the ovariectomized control rats was markedly higher than in the sham-operated controls (Table 2). Caffeic, *p*-coumaric and chlorogenic acids statistically significantly decreased the total cholesterol level in relation to the ovariectomized control rats; ferulic acid tended to decrease it.

The phenolic acids did not affect the serum level of triglycerides in ovariectomized rats (Table 2).

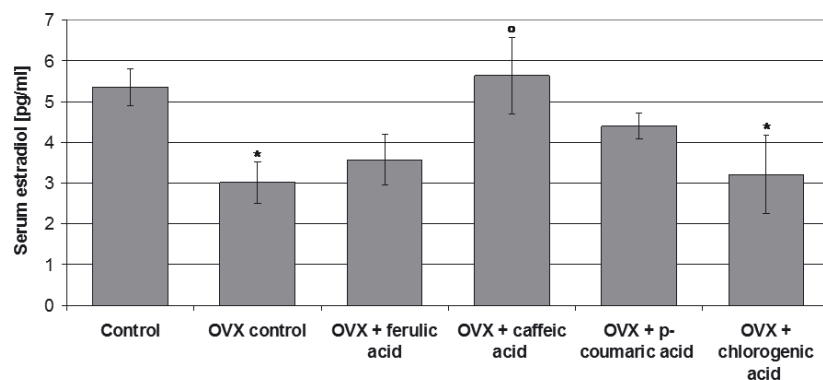
DISCUSSION

Phenolic acids are present in plant-based foods, like fruit, vegetables, grains, tea, coffee, spices, etc., and are consumed by humans every day. The estimated daily consumption of phenolic acids

ranges from 25 mg to 1 g, depending on the diet (Stalikas, 2007). In the present study, rats were administered the investigated phenolic acids at a moderate dose of 10 mg/kg daily. We did not measure the level of phenolic acids in the laboratory diet. However, the dose exceeded the dietary level, as it turned out to be sufficient to affect rat skeletal system and to decrease the body mass gain (Folwarczna *et al.*, 2009; Zych *et al.*, submitted).

The body mass gain-decreasing effect of the phenolic acids was observed both in non-ovariectomized and ovariectomized rats. Those observations are consistent with previous reports indicating possible usefulness of phenolic compounds in the treatment of obesity (for review see Hsu & Yen, 2008).

The decrease in the body mass gain in ovariectomized rats was accompanied by a decrease in the total cholesterol level. The cholesterol-lowering effects of the investigated compounds have already been demonstrated. They were studied in various

**Figure 1. Effects of natural phenolic acids on serum estradiol level in ovariectomized (OVX) rats.**

The phenolic acids (10 mg/kg p.o. daily) were administered for 4 weeks. Results are presented as means ± S.E.M. * $P < 0.05$, statistically significantly different from the sham-operated control group. ° $P < 0.05$, statistically significantly different from the OVX control group.

experimental rodent models and reported for: ferulic (Kim *et al.*, 2003; Sri Balasubashini *et al.*, 2003, Jung *et al.*, 2007; Ardiansyah *et al.*, 2008), caffeic (Lee *et al.*, 2001; Lafay *et al.*, 2005), and *p*-coumaric acid (Sharma, 1979; Zang *et al.*, 2000; Lee *et al.*, 2003).

Phenolic acids are natural phenolic compounds; another group of natural phenolic compounds are flavonoids. Both phenolic acids and flavonoids may have beneficial effects in fighting obesity (Hsu & Yen, 2008). Some flavonoids (for example isoflavones of soy: genistein and daidzein) are classified as phytoestrogens, since they exert their activity through estrogen receptors (Setchell & Lydeking-Olsen, 2003).

Estrogen deficiency in ovariectomized rats leads to increased body mass gain and this effect is reversed by supplementation of estradiol (Liang *et al.*, 2002; Meli *et al.*, 2004; Clegg *et al.*, 2006). In contrast to some flavonoids, there are very few reports connecting the effects of natural phenolic acids with estrogenic mechanisms. In the present study we demonstrated that natural phenolic acids, caffeic and *p*-coumaric acids, increased estrogen levels in ovariectomized rats. It should be pointed out that these phenolic acids had the strongest effect on the body mass gain in estrogen-deficient rats. The increases in serum estradiol levels are consistent with the decreases of the thymus mass, increased due to estrogen deficiency, which were induced by caffeic and *p*-coumaric (but also chlorogenic) acids in the ovariectomized rats. Also, the investigated phenolic acids exerted some favourable effects on the skeletal system in rats with estrogen deficiency-induced osteoporosis (Sassa *et al.*, 2003; Yamaguchi *et al.*, 2008; Folwarczna *et al.*, 2009), although no effect on the uterus mass was observed. Taken together, the body mass gain-decreasing effect of caffeic and *p*-coumaric acids may be due to the increase in serum estradiol level.

The mechanism of the effect of caffeic and *p*-coumaric acids on serum estradiol level needs to be elucidated. To our knowledge, from among the phenolic acids, only ferulic acid has been reported to increase serum estradiol level in rats, however, the dose used in that study was very low (1 µg s.c. daily) (Sassa *et al.*, 2003). *p*-Coumaric acid has been reported to induce alteration in estrus cycle, ovarian and uterine mass and structure when administered to cyclic female mice (Pakrashi *et al.*, 1979), which may indicate its effects on estrogenic pathways. Our results obtained in ovariectomized rats seem to indicate that the phenolic acids (caffeic acid) may affect a metabolic pathway regulating estrogen release from extraovarian sources. There is also a possibility that some phenolic acids may exert selective estrogen receptor modulator-like activity, since caffeic

acid in the present study differentially affected different estrogen-dependent organs.

There are also some data on the *in vitro* estrogenic effects of phenolic acids. Ferulic acid stimulated proliferation of human breast cancer MCF7 cells in a concentration- and estrogen receptor-dependent manner; that activity was attributed to up-regulation of HER2 and estrogen receptor α expression (Chang *et al.*, 2006). On the other hand, caffeic acid was reported to non-significantly increase proliferation of MCF7 cells (Stromeier *et al.*, 2005) and chlorogenic acid did not exert estrogenic activity in MCF7/ER-luc cells (Innocenti *et al.*, 2007).

Concluding, the phenolic acids decreased the body mass gain of ovariectomized rats. In the case of caffeic acid this effect could be due to an increase in estrogen level. Results of the present study point out that there is a need for conducting more advanced studies on the estrogenic effects of phenolic acids.

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