Effects of Benzyl Glucoside and Chlorogenic Acid from *Prunus mume* on Adrenocorticotropic Hormone (ACTH) and Catecholamine Levels in Plasma of Experimental Menopausal Model Rats

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Received August 5, 2003; accepted October 14, 2003

To investigate the effectiveness of benzyl β-D-glucopyranoside (BG) and chlorogenic acid (CA), the constituents of the fruit of *Prunus mume*, for relieving tension in experimental menopausal model rats (M-rats) caused by ether stress, the effects of BG and CA on adrenocorticotropic hormone (ACTH) and catecholamine (adrenaline, noradrenaline, and dopamine) levels were examined in the plasma of M-rats. Caffeic acid, quinic acid, and rosmarinic acid, which are compounds structurally related to CA, were also examined. BG obviously recovered catecholamine levels decreased by ether stress and increased dopamine to high levels. On the other hand, CA significantly decreased the ACTH level increased by ether stress and showed the greatest effect of all compounds. These results suggest that BG and CA may contribute to relieving the tension in M-rats caused by ether stress.

Key words adrenocorticotropic hormone (ACTH) level; catecholamine level; *Prunus mume*; chlorogenic acid; benzyl β-D-glucopyranoside; ether stress

The fruit of *Prunus mume* SIEB. *et* ZUCC. (Rosaceae) has been traditionally used as medicinal food in Japan. In regard to the chemical constituents, we previously reported the isolation of benzyl β-D-glucopyranoside (BG) and chlorogenic acid (CA).[1)]

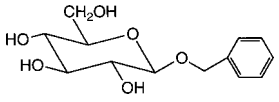
In the course of our research on the pharmacologically active constituents of the fruit of *P. mume*, we reported the inhibitory effects of BG and CA on bradykinin and prostaglandin E_2 production in the abdominal cavities of mice,[2)] and the effects of BG and CA on angiotensin-converting enzyme, aldosterone, and corticosterone levels in rat plasma.[3)] Recently, it has been reported that rosmarinic acid (RsA) is the main component involved in the antidepressive effect of Perillae Herba and in addition that it induced significant reductions in the freezing behavior of mice in the conditioned fear stress paradigm.[4,5)] Therefore, to investigate the effectiveness of BG and CA, which are structurally related to RsA, for relieving tension in experimental menopausal model rats (M-rats) caused by the ether stress generally used as forced stress in stress experiments, we examined the effects of BG and CA on adrenocorticotropic hormone (ACTH) and catecholamine (adrenaline, noradrenaline, and dopamine) levels, which participate in stress, in the plasma of M-rats. Caffeic acid (CfA), quinic acid (QnA), and RsA were also examined to compare their effectiveness with that of BG and CA.

MATERIALS AND METHODS

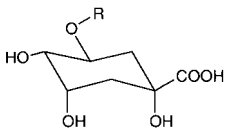
Animals M-rats were prepared as follows. Triple- or quadruple- parous rats were ovariectomized under anesthesia. After being maintained for 1 month, their follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were elevated to high levels and they were used as M-rats in this experiment. The rats were housed in a controlled environment (22±1 °C, 53±3% humidity, lights on from 06:30 to 18:30), and food and water were given *ad libitum*.

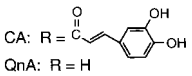
Materials Fresh fruits of *P. mume*, collected in Wakayama Prefecture in June 2002, were used for this experiment. BG and CA were obtained from the ethanolic extract of *P. mume* according to the procedure reported previously.[1)] CfA was purchased from Wako (Osaka, Japan). QnA was obtained from Acros Organics (New Jersey, U.S.A.). RsA was purchased from ICN Biomedicals Inc. (Ohio, U.S.A.).

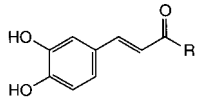
Determination of Effects on ACTH and Catecholamine Levels in M-Rat Plasma Each compound was orally administered twice daily in the morning and evening. Sixty minutes after the second administration, the M-rats were forced to inhale ether for 20 min. The inhalation apparatus was a plastic cage (13 cm high×21 cm wide×32 cm deep with a cover). Cotton was soaked in 3.0 ml of ether and placed in the cage. After inhalation for 20 min, the M-rats

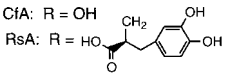


BG









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were decapitated and blood samples were obtained. Plasma was obtained from heparinized trunk blood by centrifugation (3000 rpm×15 min). Measurments of plasma ACTH and catecholamine levels were performed by SRL, Tokyo, according to the methods reported previously.[6,7]

RESULTS AND DISCUSSION

It is well known that ACTH levels are increased and catecholamine levels are decreased in plasma with stress. Adrenaline and noradrenaline levels decreased by ether stress were recovered after BG administration (Table 1). CfA and RsA recovered adrenaline levels decreased by ether stress to a greater degree than other compounds. Noradrenaline levels decreased by ether stress were recovered by CfA, QnA, and RsA administration. All compounds led to the recovery of dopamine levels and elevated them above those in control M-rats that had not inhaled ether. QnA administration showed the highest elevation of dopamine levels decreased by ether stress of all compounds tested. On the other hand, CA decreased the ACTH level increased by ether stress more markedly than other compounds. Furthermore, CA significantly increased the dopamine level decreased by ether stress. The dose-dependent effects of CA on ACTH and catecholamine levels were also examined. The administration of CA 50 mg/kg reduced ACTH to the lowest level. CA showed dose-dependent effects in the recovery of both adrenaline and noradrenaline levels decreased by ether stress. Dopamine levels decreased by ether stress were also recovered and dose dependently increased by CA. In M-rats not forced to inhale ether, no significant effects of BG and CA were seen. These data suggest that BG and CA may contribute to reducing the tension in M-rats caused by ether stress.

Table 1. Effects of BG, CfA, QnA, RsA, and CA on ACTH, Adrenaline, Noradrenaline, and Dopamine Levels in the Plasma of M-Rats

	ACTH (pg/ml)	Adrenaline (ng/ml)	Noradrenaline (ng/ml)	Dopamine (pg/ml)
M-rats	71±18.8	11.3±0.80	3.8±0.43	47.6±6.00
Ether	412±45.3***	5.5±0.56***	1.0±0.09***	29.5±3.80*
BG	421±40.8	8.6±0.69**	1.7±0.20*	53.0±13.60
CfA	365±17.2	12.9±2.34**	2.3±0.49*	58.4±7.15***
QnA	461±38.6	7.5±1.48	2.1±0.31***	79.8±5.69***
RsA	363±23.8	12.6±1.10***	2.0±0.30*	60.0±12.50*
CA 20 mg	380±19.6	6.1±1.52	1.0±0.06	38.2±3.31
50 mg	281±21.9*	6.8±1.06	1.0±0.29	63.8±10.67*
100 mg	334±21.1	8.0±0.16***	1.7±0.27	76.2±15.87***

M-rats, experimental menopausal model rats; Ether, M-rats forced to inhale ether for 20 min; BG, benzyl-β-D-glucopyranoside; CA, chlorogenic acid; CfA, caffeic acid; QnA, quinic acid; RsA, rosmarinic acid. Rats were orally administered 50 mg/kg of each compound except for CA. CA was orally administrated at doses of 20, 50, and 100 mg/kg. Each value represents mean±S.E.M. of 5 M-rats. Statistical analysis was performed using Student's *t*-test (M-rat *vs.* ether) or Dunnett's test (ether *vs.* compound). $*p<0.05$ *vs.* ether, $**p<0.02$ *vs.* ether, $***p<0.01$ *vs.* ether.

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