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Protective effect of *Aquilegia vulgaris* (L.) on APAP-induced oxidative stress in rats

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Abstract

Rats pretreated with acetaminophen (*N*-acetyl-*p*-aminophenol, APAP) (600 mg/kg b.w., p.o.) were administered with ethanol and ethyl acetate extracts as well as with isocytisoside (100 mg/kg b.w., p.o.) obtained from *Aquilegia vulgaris* (L.) (Ranunculaceae) herb. The substances tested decreased enzymatic, non-enzymatic and uninduced microsomal lipid peroxidation (LPO) in the liver of rats treated with APAP by 18–48%. Activity of the antioxidant enzymes in the liver inhibited by APAP was increased in the majority of groups after administration of the substances tested: catalase (CAT) by 55%, glutathione peroxidase (GPx) by 50%, glutathione reductase (GR) by 35% and glutathione *S*-transferase (GST) by 60%.

Hepatic glutathione level depleted by APAP was only slightly increased by the substances tested. The cytochrome P450 contents, and the activities of NADPH–cytochrome P450 reductase and two monooxygenases were not affected by the extracts and isocytisoside. It can be concluded that the protective ability of the substances tested in APAP-induced liver injury is mediated by amelioration of microsomal lipid peroxidation and restoring antioxidant enzymes activity. Inhibition of enzymes responsible for metabolic activation of APAP is not involved in this process.

Keywords: *Aquilegia vulgaris*; Antioxidant enzymes; Microsomal lipid peroxidation; Reduced glutathione

1. Introduction

There is increasing interest in the antioxidants of natural origin because they could suppress the oxidative damage of a tissue by stimulating the natural defence system. These substances, e.g., flavonoids and spice principles can serve as chemopreventive agents ameliorating the toxicity caused by certain drugs and environmental chemicals or in disease states involving oxidative stress.

Aquilegia vulgaris (L.) (Ranunculaceae), syn.: columbine, is a perennial herb indigenous in central and southern Europe. Decoction from leaves and stems of *Aquilegia vulgaris* has been used in folk medicine against liver and bile duct disorders, especially for the treatment of jaundice, and chronic skin inflammation. The herb is a component of the immunostimulating preparation Padma 28 and homeopathic drugs (PDR for Herbal Medicines, 2000). Phytochemical studies of *Aquilegia vulgaris* showed the presence of cyanogenic compounds, tannins, anthocyanins (Hänsel et al., 1992) and cycloartane derivatives showing immunosuppresive properties (Nishida et al., 2003).

We have isolated and identified several flavonoids (Bylka and Matławska, 1997a,b; Bylka, 2001; Bylka et al., 2002) and phenolic acids (Drost-Karbowska et al., 1996) in aerial parts

Abbreviations: ADP, adenosine diphosphate; APAP, *N*-acetyl-*p*-aminophenol; CAT, catalase; CDNB, 1-chloro-2,4-dinitrobenzene; DPPH, diphenyl-*p*-picrylhydrazyl; GPx, glutathione peroxidase; GSH, reduced glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase; LPO, lipid peroxidation; NADPH, nicotinamide adenine dinucleotide phosphate reduced; NAPQI, *N*-acetyl-*p*-benzoquinoneimine; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances

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Table 1
Effect of *Aquilegia vulgaris* extracts and isocytisoside on microsomal lipid peroxidation and hepatic glutathione in APAP-treated rats

Treatment	Lipid peroxidation (nmol TBARS/mg protein)				GSH (μmol/g tissue)
	Fe^{3+}/ADP/NADPH	CCl_4/NADPH	Fe^{2+}/ascorbate	Uninduced	
APAP	77.8 ± 14.6[a]	53.2 ± 9.8[a]	53.3 ± 6.6	2.86 ± 0.39	2.5 ± 0.5[a]
APAP + IST	55.8 ± 9.3[b]	46.7 ± 8.4	38.3 ± 5.1[b]	2.05 ± 0.38[b]	3.3 ± 0.5
APAP + EAE	48.9 ± 8.5[b]	40.1 ± 6.6[b]	37.4 ± 6.1[b]	1.46 ± 0.20[b]	3.6 ± 0.6[b]
APAP + EE	63.4 ± 11.0[b]	41.1 ± 5.4[b]	30.8 ± 4.2[b]	1.65 ± 0.33[b]	3.2 ± 0.5
APAP + α-toc	56.3 ± 10.9[b]	40.4 ± 5.5[b]	34.4 ± 4.9[b]	1.51 ± 0.25[b]	3.3 ± 0.6
IST	51.2 ± 9.0	33.5 ± 4.7	34.0 ± 6.0	1.88 ± 0.34[a]	5.1 ± 1.0
EAE	42.8 ± 6.4[a]	22.6 ± 4.7[a]	35.2 ± 5.1	1.43 ± 0.21[a]	5.9 ± 1.0
EE	44.3 ± 7.0[a]	23.6 ± 4.2[a]	32.5 ± 4.2[a]	1.47 ± 0.20[a]	5.7 ± 0.9
Control	61.4 ± 5.5	39.8 ± 5.8	43.3 ± 5.5	2.37 ± 0.38	4.8 ± 0.7

Results are mean ± S.D., $n = 8$. Control rats were administered vehicle only APAP, acetaminophen; IST, isocytisoside; EE, ethanol extract; EAE, ethyl acetate extract; α-toc, α-tocopherol.

[a] Significantly different from control, $p \leq 0.05$.

[b] Significantly different from APAP-treated group, $p \leq 0.05$.

of the plant as well as alkaloids in roots (Szaufer-Hajdrych et al., 1998). The predominant compound was 4′-methoxy-5,7-dihydroxyflavone 6-*C*-glucopyranoside (isocytisoside) (Bylka and Matławska, 1997a). Our previous investigation has demonstrated that ethanol extract (EE) of *Aquilegia vulgaris* and isocytisoside could protect against hepatotoxicity induced by carbon tetrachloride in rats as assessed by inhibition of transaminases and sorbitol dehydrogenase leakage to serum and by histopathological examination (Adamska et al., 2003). The hepatoprotective activity of natural substances is often associated with their capability of suppressing the effects of oxidative damage. *Aquilegia vulgaris* is rich in compounds known to be strong antioxidants, therefore, it could be expected that this very property is, at least in part, responsible for hepatoprotection observed in the previous experiment. Acetaminophen (APAP) poisoning is one of the most widely used in vivo experimental models to induce liver damage. APAP toxicity is known to be mediated by biotransformation in the liver by the microsomal P450 system to the highly reactive *N*-acetyl-*p*-benzoquinoneimine (NAPQI). At therapeutic doses, this metabolite is detoxified by reduced glutathione (GSH). Following toxic doses of APAP, GSH is depleted and the metabolite covalently binds to cellular macromolecules resulting in liver injury. Another theory states that NAPQI is an oxidising agent that depletes the cell of GSH, a cellular protectant against reactive oxygen species (ROS), thus leading to oxidative stress. There is much evidence to substantiate both theories and the question may be to what extent each plays a role in APAP toxicity (Gibson et al., 1996). The present study was undertaken to evaluate the potential protective effect of extracts and isocytisoside isolated from *Aquilegia vulgaris* on APAP-induced hepatotoxicity and to elucidate the mechanisms underlying these effects in rat. Theoretically, the hepatoprotection by *Aquilegia vulgaris* could be due to alterations in disposition and biotransformation of hepatotoxicant, alterations in cellular detoxifying mechanisms, as well as cellular response and regenerating processes (Mehendale et al., 1994). We attempted to examine two major possibilities: (1) whether *Aquilegia vulgaris* protects against hepatotoxicity of APAP by enhancing cellular defence mechanisms (2) whether *Aquilegia vulgaris* decreases the bioactivation of APAP by suppressing P450 enzymes. The parameters analysed included reduced glutathione content, microsomal lipid peroxidation (LPO),

Table 2
Effect of *Aquilegia vulgaris* extracts and isocytisoside on antioxidant and related enzymes in APAP-treated rats

Treatment	GPx (U min^{-1} mg^{-1} protein)	GR (nmol NADPH min^{-1} mg^{-1} protein)	GST (nmol CDNB min^{-1} mg^{-1} protein)	SOD (U mg^{-1} protein)	CAT (U mg^{-1} protein)	DT-diaphorase (nmol DCIP min^{-1} mg^{-1} protein)
APAP	43.1 ± 6.8[a]	17.0 ± 2.4	197.6 ± 31.2[a]	4.12 ± 0.78	26.2 ± 5.0[a]	47.5 ± 6.1[a]
APAP + IST	49.1 ± 7.5	22.3 ± 3.2[b]	272.3 ± 40.4[b]	4.39 ± 0.66	41.1 ± 7.2[b]	46.7 ± 6.3
APAP + EAE	67.3 ± 6.2[b]	23.6 ± 3.5[b]	363.0 ± 42.2[b]	5.10 ± 0.73[b]	42.5 ± 4.9[b]	38.4 ± 6.1
APAP + EE	62.6 ± 8.2[b]	16.2 ± 2.6	209.9 ± 32.4	4.20 ± 0.60	39.6 ± 4.6[b]	40.3 ± 7.4
APAP + α-toc	69.1 ± 9.0[b]	20.9 ± 4.0	262.4 ± 45.1[b]	3.89 ± 0.72	35.7 ± 4.2[b]	39.8 ± 6.8
IST	62.1 ± 8.4	21.4 ± 3.6	316.5 ± 28.0	5.00 ± 0.91	55.0 ± 5.3	36.0 ± 5.2
EAE	72.8 ± 3.3	22.5 ± 3.2	329.8 ± 20.6	6.32 ± 1.10[a]	51.8 ± 8.0	35.2 ± 6.1
EE	64.0 ± 9.5	20.5 ± 2.8	318.4 ± 36.8	6.40 ± 1.00[a]	50.0 ± 6.9	37.6 ± 6.7
Control	65.6 ± 7.3	20.3 ± 2.1	317.5 ± 18.5	4.88 ± 0.81	54.3 ± 8.2	30.4 ± 4.9

Results are mean ± S.D., $n = 8$. Control rats were administered vehicle only APAP, acetaminophen; IST, isocytisoside; EE, ethanol extract; EAE, ethyl acetate extract; α-toc, α-tocopherol.

[a] Significantly different from control, $p \leq 0.05$.

[b] Significantly different from APAP-treated group, $p \leq 0.05$.