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

Małgorzata Kujawska, Jadwiga Jodynis-Liebert, Małgorzata Ewertowska & Teresa Adamska
Department of Toxicology, Poznan University of Medical Sciences, Dojazd 30, 60-631 Poznan, Poland
and
Irena Matlawska & Wiesława Bylka
Department of Pharmacognosy, Poznan University of Medical Sciences, Święcickiego 4, 60-781 Poznan, Poland

Received 24 January 2007; revised 17 May 2007

The ethyl ether extract of *A. vulgaris* inhibited *in vitro* microsomal lipid peroxidation (IC$_{50}$ 58.8 µg/ml) and showed moderate ability to scavenge superoxide radicals and to chelate iron ions. The extract (100 mg/kg body weight, po) decreased uninduced and enzymatic microsomal lipid peroxidation in the liver of male rats pretreated with CCl$_4$ (1 ml/kg body weight) by 27 and 40%, respectively. Activity of antioxidant and related enzymes (catalase and glucose-6-phosphate dehydrogenase) inhibited by CCl$_4$ was significantly restored after administration of the extract. The extract itself significantly enhanced superoxide dismutase activity. There was no effect of the extract on hepatic glutathione level and cytochrome P450 content, both were decreased by CCl$_4$. Neither CCl$_4$ nor the tested extract affected activities of NADPH-cytochrome P450 reductase and two monoxygenases, aniline hydroxylase and aminopyrine N-demethylase. It can be concluded that the protective effect of the *A. vulgaris* extract in CCl$_4$-induced liver injury is mediated by inhibition of microsomal lipid peroxidation and restoring activity of some antioxidant and related enzymes.

Keywords: Antioxidant enzymes, *Aquilegia vulgaris*, CCl$_4$, Glutathione, Microsomal lipid peroxidation

Liver diseases remain one of the serious health problems. In absence of an effective treatment in modern medicine, efforts are being made to find out suitable herbal drugs. Hepatoprotective agents of natural origin have attracted special interest, and numerous medicinal plants and their formulations are used for liver disorders in folk medicines.

*Aquilegia vulgaris* (L.) (Ranunculaceae), locally called Columbine, is a perennial herb widely distributed throughout Eurasia and locally in North Africa. It is also found in the eastern USA. Decoction from leaves and stems of *A. vulgaris* has been used in folk medicine against liver and bile duct disorders, especially for the treatment of jaundice, scurvy, neurosis, dermatitis and as a diaphoretic agent. The herb is a component of the immunostimulating preparation Padma 28 and other homeopathic drugs.$^1$ Phytochemical studies of *A. vulgaris* showed the presence of cyanogenic compounds, tannins, anthocyanins$^2$ and cycloartane derivatives showing immunosuppressive properties$^3$.

Several flavonoids$^{4,5}$ and phenolic acids$^6$ have been isolated and identified from the leaves, stems and flowers as well as alkaloids from roots$^7$. Hepatoprotective effect of isocytisoside (4′-methoxy-5,7-dihydroxyflavone 6-C-glucopyranoside) isolated from the plant and two extracts of *A. vulgaris* in rats have been reported$^{8,9}$. Ethanol extract and isocytisoside protect against hepatotoxicity induced by CCl$_4$ as assessed by inhibition of transaminases and sorbitol dehydrogenase leakage to serum and by histopathological examination$^8$. Ethanol extract, ethyl acetate extract and isocytisoside obtained from *A. vulgaris* attenuated oxidative stress induced by acetaminophen in rats. The substances tested decreased microsomal lipid peroxidation and increased activity of antioxidant enzymes and glutathione level in the liver$^9$.

The present study has been undertaken to evaluate the potential hepatoprotective effect of the ethyl ether extract of *A. vulgaris* in rats pretreated with CCl$_4$, and to elucidate the mechanism of such activity. Two kinds of experiments were performed: under *in vitro* tests effect on microsomal lipid peroxidation, free radical scavenging ability and iron chelating activity...
were done. Protective effect of the extract in animals treated with CCl₄ was assessed by the measurement of the level of hepatic microsomal lipid peroxidation, reduced glutathione, the activities of hepatic antioxidant enzymes and some drug metabolizing enzymes in the liver.

Materials and Methods

**Chemicals and plant material**—The chemicals used were purchased from Sigma Chemical Co., USA. *A.vulgaris* stems and leaves were collected from the Botanical Garden of A. Mickiewicz University, Poznań, Poland during June 1999. A voucher specimen is deposited in the author’s laboratory (No. KF 1261999). The ethyl ether subextract was prepared as described by Bylka et al.10.

**Phytochemical analysis**—The extract was analysed by TLC as described previously and the following phenolic acids were identified: caffeic, ferulic, p-coumaric, protocatechuic, vanillic, sinapic, chlorogenic and p-hydroxybenzoic.6 Isocytisoside (4'-methoxy-5,7-dihydroxy flavone 6-C-glucopyranoside) was identified by UV and 1H NMR, 13C NMR analysis5. Quantitative analysis of isocytisoside and phenolic acids was performed by HPLC method. Lachom-Merck chromatograph equipped with DAD detector and Zorbax SB-C18 column (250 × 4.6mm; 5μm) was used. The mobile phase (flow rate 1ml/min) was methanol-water-formic acid (25:75:1 v/v) for phenolic acids analysis and (40:60:1 v/v) for isocytisoside analysis. The standard curves for phenolic acids and for isocytisoside were made in the range 2-12μg. The content of isocytisoside in the extract was 1.35%, the content of phenolic acids was as follows: protocatechuic acid 0.20%, p-coumaric acid 0.03% and vanillic acid 0.03%.

**In vitro tests**

Inhibition of microsomal lipid peroxidation stimulated with Fe²⁺/ascorbate (non-enzymatic) and Fe³⁺/ADP/NADPH (enzymatic) was assayed by thiobarbituric acid reactive substances (TBARS) test11. IC₅₀ was calculated from the concentration-effect regression line. Liver microsomes of male Wistar rats were prepared by standard procedure (differential ultracentrifugation).

Superoxide radical scavenging activity was determined on the basis of the reaction of nitroblue tetrazolium and superoxide radical generated in the hypoxanthine/xanthine oxidase system. The scavenging ability was calculated by comparison with control sample. Superoxide dismutase was used as a reference scavenger12.

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging ability was tested by the measurement of the absorbance of the reaction mixture containing DPPH radical and extract tested, as described by Muna et al13. The decrease of the absorbance was a measure of the scavenging activity. α-Tocopherol was used as a reference substance.

Hydroxyl radical scavenging activity—hydroxyl radicals generated in Fenton reaction cause a degradation of the deoxyribose and formation of TBARS. Any compound which can scavenge hydroxyl radical causes decrease in TBARS formation. Mannitol, a well known •OH scavenger was used as a positive control14.

Iron chelation ability was assayed by ferrozine assay15. Ferrozine binds Fe²⁺ forming complex with a high extinction coefficient. Any compound which chelates Fe²⁺ causes the decrease in the absorbance of this complex. Results were expressed as percent of the activity of EDTA, a reference compound binding Fe²⁺ at 100%.

The content of phenol groups was determined with Folin-Ciocalteu reagent16.

**Experimental design**

Male Wistar rats (240±10g) were divided randomly into 5 groups (I-V), of 8 animals each. The rats were housed in an animal facility at 22°±1°C with 12hr light–dark cycle, controlled humidity and circulation of air. The substances tested and CCl₄ were administered intragastrically in the mixture of water and olive oil (1:1 v/v) with a drop of Tween 20. Animals in groups I-III were given CCl₄ at a dose 1 ml/kg body weight. After 4 hr these groups were treated as follows: group I was given vehicle, group II – ethyl ether extract, and group III – α-tocopherol. Groups IV and V were given vehicle and after 4 hr group IV was administered ethyl ether extract and group V – which served as control – vehicle again. The ethyl ether extract and α-tocopherol were given at a dose 100 mg/kg body weight. α-Tocopherol, a model antioxidant, was used as a positive control. After 19 hr of the first treatment animals were sacrificed by decapitation. The livers were removed, perfused with ice-cold 1.15% KCl and homogenised in buffered sucrose solution (TRIS, pH 7.55). Microsomal and cytosol fractions were prepared by differential centrifugation according to the standard procedure. Protein concentration in the fractions, was
determined using Folin-Ciocalteu reagent. Liver homogenate for glutathione determination was prepared in phosphate buffer, pH 7.4.

The experiment was performed according to The Local Animal Ethics Committee guidelines for animal experimentation and permission from Local Animal Ethics Committee was obtained before the experiment.

**Biochemical assays**

**Lipid peroxidation assay**—Microsomal lipid peroxidation (LPO) in the liver was assayed in three different experimental systems: (i) Fe^{3+}/ADP/NADPH–stimulated peroxidation-enzymatic, (ii) Fe^{2+}/ascorbate-stimulated peroxidation-non enzymatic and (iii) uninduced peroxidation. The level of lipid peroxidation was evaluated by measuring thiobarbituric acid reactive substances (TBARS). The results were expressed in nmol TBARS per mg protein.

**Reduced glutathione, antioxidant and related enzymes assays**—GSH level was assayed in the liver homogenate prepared in phosphate buffer (pH 7.4) by the method of Sedlak and Lindsay with Ellman’s reagent. The results were expressed in µmol/g tissue. Glutathione peroxidase (GPx) activity was determined according to Mohandas et al. Hydrogen peroxide was used as a substrate. The disappearance of NADPH at 340nm was a measure of enzyme activity, which was expressed as nmol NADPH oxidized/min/mg protein.

Glutathione reductase (GR) was assayed by measuring NADPH oxidation at 340nm using oxidized glutathione as a substrate. The activity was expressed in nmol NADPH oxidized/min/mg protein.

Catalase (CAT) activity was determined according to Beer and Sizer. The rate of H_{2}O_{2} reduction was a measure of CAT activity. One unit of CAT reduces 1µM of H_{2}O_{2}/min. The results were expressed in units/mg protein.

Superoxide dismutase (SOD) activity was determined by the method of Sun and Zigman. Inhibition of spontaneous epinephrine oxidation was a measure of SOD activity. For calculation, the standard curve of SOD activity was used. The results were expressed in units/mg protein.

Glutathione s-transferase (GST) activity measurement was based on the spectrophotometric determination of 1-chloro-2,4-dinitrobenzene (CDNB) conjugate formed in a GSH coupled reaction. The activity was expressed as nmol CDNB conjugated with GSH/min/mg protein.

Glucose-6-phosphate dehydrogenase (G6PDH) activity was determined according to Orten et al. The increase in absorbance at 340 nm, caused by the reduction of NADP is a measure of the catalytic activity of the enzyme.

**Microsomal enzymes assay**—Cytochrome P450 content was assayed by the method of Omura and Sato based on the carbon monoxide difference spectra of dithionite-reduced microsomes. NADPH-cytochrome P450 reductase activity was measured using cytochrome C as an electron acceptor in the presence of NADPH. Aminopyrine n-demethylase activity was determined by measuring the amount of formaldehyde formed using the Nash reagent.

Aniline hydroxylase activity was assayed by the spectrophotometric determination of p-aminophenol, produced as a result of aniline hydroxylation. Gamma-glutamyltranspeptidase (GGTP) activity was assayed by the spectrophotometric determination of 5-amino-2-nitrobenzoic acid. The inhibiting activity of α-tocopherol was much greater than those of the extract: 9-fold (enzymatic LPO) and 17-fold (nonenzymatic LPO) (Table 1).

The tested extract appeared to be rather weak •OH scavenger, since it decreased deoxyribose degradation by 17% at a concentration of 100µg/ml (Table 1). Similar scavenging activity of the extract, at the same concentration, was observed towards DPPH radical-23% (Table 1).

The content of phenolic groups in the extract was 18.0 ± 0.9 µM/mg.
Iron chelation ability, expressed as a percent of EDTA iron chelation was 35.1% ± 2.1.

**In vivo assays**

In enzymatic \( \text{Fe}^{3+}/\text{ADP}/\text{NADPH} \) stimulated microsomal lipid peroxidation, TBARS level was significantly increased (by 40%) in rats that were administered \( \text{CCl}_4 \), when compared to controls. The ethyl ether extract caused a significant decrease in the TBARS level by 40% in comparison to those in \( \text{CCl}_4 \)-intoxicated group. \( \alpha \)-Tocopherol effect was similar, causing 58% reduction of TBARS level (Table 2).

Non-enzymatic LPO stimulated by \( \text{Fe}^{2+}/\text{ascorbate} \) was more affected by \( \text{CCl}_4 \) treatment, five-fold elevation of TBARS level was recorded. However, single oral administration of extract tested did not result in a significant alteration of TBARS level, when compared to the \( \text{CCl}_4 \)-treated rats. More effective was \( \alpha \)-tocopherol that inhibited TBARS formation, by 95% to the level lower than that in control rats (Table 2).

Uninduced lipid peroxidation was significantly increased (150%) after \( \text{CCl}_4 \) administration. The extract tested decreased (27%) the level of TBARS insignificantly. Administration of \( \alpha \)-tocopherol caused a significant reduction of TBARS level (44%), when compared to that in control rats.

The extract tested alone caused the increase in microsomal LPO in all assays, especially in \( \text{Fe}^{2+}/\text{ascorbate} \) stimulated LPO (up to 6-fold), and 2-fold elevation in two other assays (Table 2).

A significant decrease (18%) in concentration of reduced glutathione, was observed in the \( \text{CCL}_4 \)-treated rats. Neither the extract tested nor \( \alpha \)-tocopherol affected this parameter as compared to \( \text{CCL}_4 \)-treated and control rats (Table 2).

All enzymes involved in glutathione metabolism in the liver were inhibited by \( \text{CCL}_4 \) treatment. The response of GPx and GR was significant, 35% and 41% inhibition, respectively, whereas inhibition of GST was weaker (19%) and insignificant. Administration of the extract tested and \( \alpha \)-tocopherol to \( \text{CCL}_4 \)-treated rats did not cause any noticeable alterations in any of these parameters. However, the extract alone significantly reduced activity of GPx and GR by 43% and 47%, respectively (Table 3).

A significant decrease in SOD activity (by 33%) was observed in the liver of \( \text{CCL}_4 \)-treated rats as compared to that in controls. Neither extract tested nor \( \alpha \)-tocopherol significantly changed the activity of SOD as compared to \( \text{CCL}_4 \)-treated animals. However, the extract tested alone caused significant increase, by 42%, in SOD activity.
CAT activity was significantly reduced in rats treated with CCl₄, by 35%, as compared to that in controls. Both, the extract and α-tocopherol caused equal elevation of CAT activity, by 130%, exceeding the level observed in the control group (Table 3).

Hepatic G-6-PDH activity in rats treated with CCl₄ was significantly lower than that in control rats. The extract and α-tocopherol markedly attenuated this decrease by 57% and 88%, respectively. The extract alone caused significant decrease in this enzyme activity (Table 3).

The administration of CCl₄ elevated the activity of hepatic GGTP in rats. The treatment with the extract decreased the enzyme activity by 22%. The effectiveness of the extract was comparable to α-tocopherol, a potent antioxidant used as a reference. The extract alone did not change GGTP activity (Table 3).

The effect of the extract on total cytochrome P450 content was also examined. Treatment with CCl₄ significantly depleted hepatic cytochrome P450 content by 75% as compared to that in control rats. The extract tested caused a slight and insignificant elevation of the enzyme content. However, in rats treated with the extract alone cytochrome P450 content was significantly increased (108%), as compared to that in control rats (Fig. 1).

Neither CCl₄ nor the extract affected the activity of NADPH-cytochrome P450 reductase, aminopyrine N-demethylase and aniline hydroxylase (data not shown).

**Discussion**

Phenolic acids, besides isocytisoside, are the main active components of the ethyl ether extract of A. vulgaris. Previously tested ethanol and ethyl acetate extracts did not contain this class of compounds.

---

**Table 3—Effect of A. vulgaris ethyl ether extract on antioxidant and related enzymes in CCl₄-treated rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>SOD U x mg⁻¹ protein</th>
<th>CAT U x mg⁻¹ protein</th>
<th>GPx nmol NADPH x min⁻¹ x mg⁻¹ protein</th>
<th>GR nmol NADPH x min⁻¹ x mg⁻¹ protein</th>
<th>GST nmol CDNB x min⁻¹ x mg⁻¹ protein</th>
<th>G-6-PDH U x mg⁻¹ protein</th>
<th>GGTP mU x mg⁻¹ protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>7.2±0.4</td>
<td>21.8±1.6</td>
<td>425±49</td>
<td>81.6±5.6</td>
<td>7660±65</td>
<td>20.60±1.85</td>
<td>5.3±0.3</td>
</tr>
<tr>
<td>CCl₄</td>
<td>4.8±0.3ᵃ</td>
<td>14.3±1.2ᵃ</td>
<td>276±10ᵇ</td>
<td>48.3±3.3ᵃ</td>
<td>623±44</td>
<td>8.47±1.02ᵃ</td>
<td>7.7±0.8ᵃ</td>
</tr>
<tr>
<td>CCl₄ + extract</td>
<td>4.0±0.6</td>
<td>32.7±1.3ᵇ</td>
<td>283±17</td>
<td>42.0±2.8</td>
<td>727±61</td>
<td>13.32±1.81ᵇ</td>
<td>6.0±1.0ᵇ</td>
</tr>
<tr>
<td>CCl₄ + α-toc</td>
<td>5.7±1.6</td>
<td>32.4±4.6ᵇ</td>
<td>262±25</td>
<td>42.6±5.6</td>
<td>677±92</td>
<td>15.97±1.37ᵇ</td>
<td>5.0±0.4ᵇ</td>
</tr>
<tr>
<td>Extract</td>
<td>10.2±1.2ᵃ</td>
<td>15.7±3.3ᵃ</td>
<td>241±29ᵇ</td>
<td>43.0±4.3ᵃ</td>
<td>658±18</td>
<td>15.82±1.86ᵃ</td>
<td>5.7±0.8</td>
</tr>
</tbody>
</table>

P values: ≤0.05; significantly different fromᵃ control;ᵇCCl₄-treated group

---

Fig. 1—Effect of A. vulgaris ethyl ether extract on cytochrome P450 content in CCl₄-treated rats [Values are mean ± SD from 8 animals in each group. P≤0.05; α-toc: α-tocopherol]

Therefore, it was interesting to assess what was the potential antioxidant activity of this extract.

*In vitro tests*—NADPH–dependent LPO is catalysed by cytochrome P450 in conjunction with NADPH–cytochrome P450 reductase. As a result, free radicals such as O₂⁻ and ROO⁻ are generated. The mechanism of inhibition of enzymatic LPO may be ascribed both to inhibition of cytochrome P450 and its reductase, and free radicals scavenging. In our study, enzymatically-driven LPO was inhibited more strongly by the extract tested than non-enzymatic LPO, a solely free-radical process. However, the *in*
vivo experiment showed that the extract itself cause a substantial increase in hepatic cytochrome P450 content. Hence, the inhibition of cytochrome P450 cannot be considered as a mechanism of LPO decrease in vitro. The extract can be classified as a chain-breaking antioxidant since it inhibited NADPH–dependent LPO, the process involving generation of a great amount of ROO• radicals. It was mentioned above, the enzymatic, NADPH-dependent LPO is catalyzed by the NADPH-cytochrome P450 reductase and propagated by cytochrome P450 with the generation of free radicals i.e. O2•− and ROO•. It was observed that the extract tested did not decrease the activity of NADPH-cytochrome P450 reductase in CCl4-treated rats. This may suggest that the mechanism of inhibition of NADPH-dependent LPO by the A. vulgaris extract could be associated with their radical scavenging ability. The present in vitro studies confirmed this suggestion. Ethyl ether extract of A. vulgaris was found to scavenge effectively, superoxide anion and showed iron chelating ability. Hence, the mechanism of inhibition of iron-stimulated LPO observed in the present study may involve the formation of complexes between iron and components of the extracts. This would prevent the generation of free radicals and thus inhibit LPO.

Surprisingly, the extract tested alone caused the increase in microsomal LPO in all assays. It seems that this increase can be associated with a marked (95%) elevation of hepatic P450 content. It is known that in vivo cytochrome P450 can function as a peroxidase using lipid peroxides as substrates, which is a NADPH-independent process that results in the formation of peroxyl, alkoxyl and hydroxyl radicals. Moreover, P450 is a source of oxygen radicals, which in the presence of non-hem iron can initiate peroxidation. In animals challenged with CCl4, the content of cytochrome P450 was substantially reduced and in such conditions the extract could inhibit enzymatically-driven lipid peroxidation to some degree.

The discrepancy between the effect of the extract on LPO in vitro and in vivo could be explained by the possible biotransformation of components of the extract to metabolites showing prooxidant properties. Such conversions may be facilitated by the elevated content of hepatic cytochrome P450.

Reduced glutathione—Reduced glutathione is an intracellular reductant which plays major role in detoxification of xenobiotics and their metabolites. Electrophilic compounds form conjugates with GSH either spontaneously or enzymatically in reactions catalyzed by glutathione s-transferase and the conjugates are usually excreted from the cell. GSH
serves an antioxidant function too. In the presence of glutathione peroxidase, GSH reduces endogenously produced H$_2$O$_2$ and organic peroxides.$^{31}$

The decrease in GSH content in CCl$_4$-treated rats may be explained by increased utilization of GSH for removal of ROS. It has been reported that GSH in hepatic cells is depleted in 24 hr following oral administration of CCl$_4$ and that this depletion is associated with a decrease in hepatic activities of antioxidant enzymes. In turn, depletion of reduced glutathione by CCl$_4$ ultimately resulted in lipid peroxidation, which is believed to be responsible in part for subsequent hepatocellular damage.$^{32-34}$

Neither A. vulgaris extract nor α-tocopherol prevented the depletion of GSH caused by CCl$_4$. Thus, it can be concluded that antioxidant activity is not essential in restoring the GSH level. This observation was confirmed by Miccadei et al.$^{35}$ They added model antioxidants N,N′-diphenyl-p-phenylenediamine and deferoxamine to hepatocyte cultures in which GSH depletion was evoked by dinitrofluorobenzene or diethyl maleate. They found that the antioxidants used inhibited lipid peroxidation and prevented the cell death. However, they did not restore the GSH level. Similar effects were observed in rats administered with iron chelator, deferoxamine after pretreatment with prooxidants, bromobenzene and allyl alcohol.$^{36}$

It has been demonstrated that the γ-glutamyl transpeptidase (GGTP) induction can occur as a protective adaptation that allows the cell an access to more cysteine and thereby increases intracellular glutathione, which is protective against oxidative stress.$^{37}$ If GSH level is decreased, as has been observed in the CCl$_4$-treated rats in the present experiment, the GGTP activity is induced, and probably stimulates GSH biosynthesis. Similar response was observed in T-2 toxin treated chickens. In the birds treated simultaneously with a potent antioxidant lycopene and mycotoxin a reduction in the enzyme activity was observed.$^{38}$ A. vulgaris extract and α-tocopherol treatment resulted in a significant decrease in GGTP levels. Similar effects were observed in rats administered 1,2-dimethylhydrazine hydrochloride after pretreatment with Picroliv, a natural product, containing mainly iridoid glycosides.$^{39}$ Treatment with phenolic compounds – caffeic acid phenethyl ester, butyld hydroxyanisole and butyl hydroxytoluene – was found to reduce the elevated levels of GGTP.$^{40,41}$

**Antioxidant enzymes** — When cells are exposed to oxidative stress, they increase the activity and expression of antioxidant enzymes as a compensatory mechanism to protect them from the damage induced by free radicals. In many cases, the number of free radicals generated may be so great that even the increased activity of the antioxidant enzymes is insufficient to counteract the potential damage. It was found that under heavy oxidative stress conditions, antioxidant enzymes activity was diminished because of a damage of the molecular machinery that is required to induce these enzymes.$^{42}$ Consistent with this finding, in the present experiment the activities of antioxidant enzymes, except glutathione s-transferase were reduced in rats treated with CCl$_4$. Similar results were reported by many authors who observed the decrease in antioxidant enzyme activity in the liver of rats treated with CCl$_4$ at a single oral$^{43}$, or intraperitoneal dose$^{44,45}$ and after repeated administration.$^{46-48}$

SOD, CAT and GR are known to be inactivated *in vitro* by H$_2$O$_2$, O$_2$•−, and 'OH, respectively. SOD and CAT are major antioxidant defense components that primarily catalyze the conversion of superoxide radical O$_2$•− to H$_2$O$_2$ (SOD) and decomposition of H$_2$O$_2$ to H$_2$O (CAT). H$_2$O$_2$ is normally detoxified in cells by either CAT and/or GPx. GPx catalyzes the reduction of H$_2$O$_2$ by reduced glutathione (GSH). GSH is readily oxidized to glutathione disulfide (GSSG) by the GPx reaction. GSSG can be reduced by NADPH–dependent reaction catalyzed by glutathione reductase. NADPH is an essential cofactor for the regeneration of GSH. Glucose-6-phosphate dehydrogenase is the first and rate-limiting enzyme of the pentose phosphate pathway and is regarded as a major enzyme to generate NADPH.$^{49,50}$

A decrease in SOD, CAT and GPx activity with CCl$_4$ probably results in accumulation of O$_2$•− and H$_2$O$_2$ which react with metal ions to promote additional radical generation, with the release of the particularly reactive hydroxyl radicals. Hydroxyl radicals react with lipids, DNA and proteins, caused a loss of cell integrity, enzyme function, and genomic stability.$^{49,51}$ A. vulgaris extract restored markedly, only the catalase activity reduced by CCl$_4$ pretreatment. However, treatment with the extract alone caused a significant decrease in the activity of CAT, GPx and GR. SOD was the only enzyme whose activity was induced by the extract tested. Similar effect was observed in fish administered tannic acid,
used as a model of plant-derived polyphenolic antioxidant. Varanka et al.\textsuperscript{52} reported, that tannic acid significantly inhibited antioxidant enzymes in fish, except SOD, both in vitro and in vivo. Toda et al.\textsuperscript{53} have reported that ferulic acid scavenges superoxide anion radical and inhibits lipid peroxidation induced by superoxide, and the effect of ferulic acid is similar to that of SOD. It could explain the induction of SOD activity caused by the extract tested alone.

Glucose-6-phosphate dehydrogenase (G-6-PDH) activity was markedly reduced in the rats after CCl\textsubscript{4} treatment. The present results agree with the other reports in which inhibition of G-6-PDH activity in rats challenged with CCl\textsubscript{4} was observed\textsuperscript{54,55}.

A. vulgaris extract restored significantly the activity of G-6-PDH in the CCl\textsubscript{4}-treated rats. Similar results were reported by Parvez et al.\textsuperscript{56}. They investigated the modulatory and protective effect of natural antioxidants like catechin on the toxicity of an anticancer drug, tamoxifen (TAM). It was found that antioxidants used significantly increase the activity of G6-PDH, both in the liver and kidney as compared to TAM-treated animals.

Drugs metabolizing enzymes—The metabolic activation of CCl\textsubscript{4} is thought to be mediated through CYP2E1. Hence, attempts were made to examine whether the substances tested were capable of decreasing the activation of CCl\textsubscript{4} by suppressing some phase I drug metabolizing enzymes. Total P450 content, NADPH-cytochrome P450 reductase as well as activities of two monooxygenase were determined. The activity of aniline hydroxylase is known to be mainly CYP2E1 dependent\textsuperscript{57}. Aminopyrine is used as a non specific substrate for measuring the hepatic metabolic capacity of the cytochrome P450 system\textsuperscript{58}.

In the present study, CCl\textsubscript{4} administration alone significantly decreased the content of hepatic cytochrome P450. It is known that CCl\textsubscript{4} acts as a suicide inactivator of cytochrome P450. Reactive metabolites of CCl\textsubscript{4} are responsible for this effect. The extract alone caused over 2-fold increase in cytochrome P450 content, however, it did not affect the level of the enzyme in CCl\textsubscript{4}-pretreated rats. The suggested relationships between the content of hepatic cytochrome P450 and lipid peroxidation have been discussed in earlier.

The extract tested in the present study did not affect the activities of NADPH-cytochrome P450 reductase and two monooxygenases tested in rats challenged with CCl\textsubscript{4} and in those treated with the extract alone (data not presented). Hence, it could be concluded that its protective action is not due to the inhibition of CCl\textsubscript{4} toxic metabolites generation. The present results demonstrated that the ethyl ether extract from A. vulgaris acts as an antioxidant by restoring the activity of enzymes such as CAT, G6-PDH, and inhibition of microsomal lipid peroxidation.

It could be suggested that phenolic acids and/or their metabolites are responsible for the increase in hepatic microsomal lipid peroxidation and the decrease in some antioxidant enzyme activities in rats treated with the extract alone. Previously tested extracts of A. vulgaris did not contain phenolic acids and did not cause such prooxidant effects.

Acknowledgement

This work was partly supported by a research grant of the Polish State Committee for Scientific Research (No. 0835/P05/2001/20).

References


46 Campo G M, Avenoso A, Campo S, D'Ascola A, Ferlazzo A M & Calatroni A, The antioxidant and antifibrogenic effects of the glycosaminoglycans hyaluronic acid and chondroitin-4-sulphate in a subchronic rat model of carbon tetrachloride-