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Neuropharmacological evaluation of diethylether extract and xanthones of *Gentiana kochiana*

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Abstract

Diethylether extract of aerial parts of *Gentiana kochiana* mostly consists of two tetraoxygenated xanthones: gentiacaulein (1,7-dihidroxy-3,8-dimethoxyxanthone; 76.1%) and gentiakochianin (1,7,8-trihidroxy-3-methoxyxanthone; 14.2%). The extract and these xanthones were evaluated for the CNS pharmacological activity in rodents. In vitro assays on rat brain preparations revealed insignificant interaction of the compounds with the specific dopamine and serotonin receptors or synaptosomal uptake of serotonin. However, the extract and gentiacaulein strongly inhibited rat microsomal MAO A (IC_{50}=0.22 μg/ml and 0.49 μM, respectively). Their effects on MAO B and a gentiakochianin blocking potential on both MAO enzymes were moderate. Behavioral examinations on mice showed that 10 day s.c. administration of the extract (20 mg/kg) significantly decreased immobility score in a forced swimming test and strongly inhibited ambulation and stereotypy in an open-field test. These effects resembled those induced by 10 mg/kg imipramine. The ex vivo MAO A activity in crude brain mitochondrial fraction of mice treated with 20 mg/kg of the extract was significantly elevated, whilst that outside brain nerve terminals was declined. This study suggests some antidepressant therapeutic potential of *G. kochiana*, particularly of gentiacaulein, with an ambiguity whether pharmacological mechanism could be related only to the central inhibition of MAO A.

Keywords: *Gentaiana kochiana*; Xanthones; MAO inhibitor; Antidepressant activity; Behavioral tests; Rodents

1. Introduction

Gentiana kochiana Perr. et Song. (Gentianaceae) is widespread South-European specie, growing on acidic soils at altitudes between 1700 and 3000 m (Jovanović-Dunjić, 1973). Similar to the use of some other *Gentiana* species throughout the world, root preparations of *G. kochiana* are applied in the region of Tuscany to help digestion and stimulate appetite (Baragatti et al., 2002). This herb is also employed in traditional medicine as antihypertensive, antipyretic, depurative and spasmolytic (Unicini Manganelli et al., 2000; Baragatti et al., 2002). Xanthones and xanthone glycosides are among the major identified components in *G. kochiana* (Rivaille et al., 1969). Xanthones are yellow plant pigments with a chemotaxonomic importance (Meszaros, 1994) and a number of different pharmacological effects (e.g. antitumor, antioxidant, CNS depressant or stimulant), evaluated by in vitro and in vivo studies (Ghosal et al., 1975; Peres and Nagem, 1997; Peres et al., 2000, Mahabusarakam et al., 2000; Dall'Acqua et al., 2002). They also have commercial significance, while fruit beverages made of *Garcinia mangostana*, containing over 40 of natural xanthones, have become recently popular as an alternative medicine product. The group of simple oxygenated xanthones occurs frequently in the species of genus *Gentiana* and exhibits a broad spectrum of pharmacological effects (reviewed by Peres and Nagem, 1997, and Peres et

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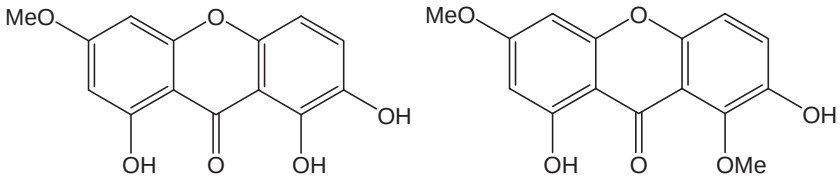

Gentiakochianin Gentiacaulein

Fig. 1. Structure of xanthones isolated from diethylether extract of *G. kochiana* aerial parts.

al., 2000). Six tetraoxygenated xanthones with hydroxy and/or methoxy groups on the C-positions: 1, 3, 7 and 8, were found in *G. kochiana* so far (Rivaille et al., 1969; Peres et al., 2000). It was recently suggested that some of these compounds are responsible for the relaxing properties and antihypertensive effects of crude root extracts of this plant (Chericoni et al., 2003). We found that the two of these xanthones, gentiacaulein and gentiakochianin, were abundant in the diethylether (Et_2O) extracts of the aerial parts of *G. kochiana* (Fig. 1). Gentiacaulein was also identified in other *Gentiana* species (Peres et al., 2000), and it was indicated as a strong reversible inhibitor of monoamine oxidase (MAO, EC:1.4.3.4) A in vitro (Suzuki et al., 1981). Although this suggests an antidepressant potential, little has been done to precisely evaluate its in vivo effects and a plausible therapeutic application.

In the present study, we have extended pharmacological in vitro analyses of two purified xanthones, gentiacaulein and gentiakochianin, and the Et_2O extract of *G. kochiana* and evaluated their interaction with rat brain synaptosomal serotonin (5-HT) reuptake, dopamine (DA) and 5-HT receptors, as well as with rat hepatic mitochondrial MAO enzymes. The extract was also examined by behavioral assays in mice, using forced swimming test (FST) to estimate antidepressive potential, and an open-field locomotor test to evaluate the impact of the extract on animal motor behavior. In addition, the brains of all animals treated with the extract for 10 days and applied in behavioral studies were analyzed for the total MAO A and B activities ex vivo.

2. Methods

2.1. Animals

All in vitro experiments were done on preparations isolated from adult Mill-Hill hooded rat males (b.w. 200–250 g). Male adult CBA mice were used for behavioral experiments. Behavioral tests were performed daily between 10 A.M. and 1 P.M. The animals were housed in groups of 5–6 per cage, under controlled conditions (room temperature 23–25 °C, 12 h light–dark cycle, food and water ad libitum) in the vivarium of the Institute for Biological Research, Belgrade. The maintenance of animals and experimental protocols were in accordance with the Official Institutional Guide for Experimental Work on Animals, adjusted to the European Communities Council Directive (86/609).

2.2. Plant material and drugs

Plant material was collected at mountain Komovi in Montenegro (at ca 2000 m) in a summer of 2002. Air-dried aerial parts of *G. kochiana* (330 g) were extracted with methanol for 48 h at room temperature. The extract was evaporated in vacuo to yield brown residue (79.2 g), which was suspended in water and re-extracted with solvents of increasing polarity: Et_2O (yield of 17.1 g), ethyl acetate and *n*-butanol. The analyses of the Et_2O extract by liquid chromatography–photodiode array detector (LC–DAD) (Menković et al., 2002) indicated the presence of xanthone aglycones. The Et_2O extract was subjected to dry column flash chromatography on silica gel using toluene with increasing amounts of ethyl acetate (10–100%) to give 30 fractions. Fractions 5. and 12. were chromatographed on Sephadex LH-20, eluting with CH_2Cl_2/methanol (1:1), to yield gentiakochianin and gentiacaulein, respectively (Rivaille et al., 1969). The relative concentrations of gentiacaulein and gentiakochianin in the Et_2O extract based on peak areas in chromatogram, obtained by high performance liquid chromatography (HPLC), were 76.1% and 14.2%, respectively. The extract and purified xanthones were dissolved in dimethyl sulfoxide (DMSO) and diluted with appropriate solutions. Maximal concentrations of DMSO for in vitro and in vivo experiments were up to 1% and 4%, respectively, i.e. within the range without significant influence on the results. Concentrations of the extract and xanthones, applied for the in vitro assays, ranged from 0.01 to 1000 μg/ml and from 0.01 μM to 1.0 mM, respectively.

The drugs used (clorgyline, pargyline, butaclamol, serotonin, tyramine, amitriptyline) were either the products of Sigma Chemical, USA, or ICN Biomedicals, USA. They were dissolved in deionized water ex tempore. All other chemicals were of the highest purity obtainable from Sigma Chemical. Radiologands were products of — Amersham, USA: ^{3}H-serotonin (46 Ci/mmol), ^{3}H-SCH23390 (91 Ci/mmol), ^{3}H-spiperone (25 Ci/mmol) ^{3}H-mesulergine (86 Ci/mmol); Perkin-Elmer, USA: ^{3}H-8OH-DPAT (129 Ci/mmol) and ^{3}H-ketanserin (88 Ci/mmol); or American Radiolab Chemicals, USA: ^{14}C-tryptamine (55 mCi/mmol).

2.3. In vitro assays

2.3.1. Monoamine oxidase assay

Partially purified mitochondria preparations of rat liver homogenates (Holt et al., 1997), used as a source of MAO enzymes, were stored in aliquots at −70 °C until assayed. The MAO activity was determined radiometrically by the modified method of Yu and Hertz (1982), using ^{14}C-tyramine as a MAO substrate. To distinguish MAO A from MAO B activity, pargyline (200 nM) and clorgyline (30 nM) were used. The experimental samples in duplicates, containing homogenized mitochondrial preparation, MAO inhibitors and different concentrations of the Et_2O extract or xanthones, were preincubated at 37 °C for 20 min in a final volume of 200 μl of 50 mM K-phosphate buffer, pH 7.5. The assay was initiated by adding 50 μl of the substrate (mixture of 500 μM radioinert *p*-tyramine and 1.0 μCi/ml ^{14}C-tyramine) and after incubation at 37 °C for 30 min the reaction was terminated with 150 μl citric acid (4.0 M). Reaction products were extracted by vigorous shaking in 1.0 ml of toluene and ethyl acetate (1:1) and radioactivity of the organic phase measured (LKB 1219 RACKBETA LSC) in liquid scintillation cocktail (PPO/POPOP/toluene). Blank values were obtained by adding citric acid before the substrate.

2.3.2. Radioligand receptor binding assays

Preparation of rat striatal and cortical membranes and in vitro binding assays were performed by standard pharmacological procedures described in Vogel (2002). Samples in duplicate containing the extract or xanthones in various concentrations, synaptosomal membranes (striatal for DA, cortical for 5-HT receptors) and appropriate radioligand, were incubated in a water bath (10 min, 37 °C). The assays on 5-HT receptors were performed in 50 mM Tris–HCl, pH 7.7, and those on DA receptors in a buffered mixture containing 120 mM NaCl, 5 mM $MgCl_2$, 2 mM $CaCl_2$ and 50 mM Tris–HCl, pH 7.5. To determine nonspecific binding to the DA and 5-HT receptors, butaclamol (1.0 μM) and serotonin (10 μM) were used, respectively. The reactions were terminated by adding ice-cold buffer. After vacuum filtration (Whatman GF-B filters) and thorough rinsing with the buffer, radioactivity remained on filters was measured using the above liquid scintillation method.

2.3.3. Synaptosomal 5-HT uptake assay

A common experimental details on preparation of rat cortical synaptosomes and mini 5-HT uptake assay were described elsewhere (Vogel, 2002). Synaptosomal solution (100 μl; 50–60 μg protein) and 80 μl of either of the herbal compounds diluted in the uptake buffer (freshly prepared Krebs–Ringer phosphate buffer, pH 7.2 containing 124 mM NaCl, 5 mM KCl, 1.3 mM $MgSO_4$, 0.75 mM $CaCl_2$, 20 mM Na_2HPO_4, 1.2 mm KH_2PO_4, 10 mM glucose, 10 μM pargyline, 25 μM iproniazide and 0.03 % ascorbic acid) were mixed and preincubated (10 min, 37 °C). Uptake reaction, initiated by adding 20 μl [^{3}H]-5-HT (500 nM) to the samples in duplicate was continued for 4 min at 37 °C and terminated by introducing 1.0 ml of ice-cold uptake buffer. It was immediately followed by vacuum filtration and two 2.5 ml rinses with the same medium. Radioactivity remained on the filters was determined as above by liquid scintillation counting. Blank values were determined by measuring samples adjuncted with 20 μM amitryptiline.

2.4. Behavioral tests

2.4.1. Forced swimming test

A modification (Detke et al., 1997) of the original FST (Porsolt et al., 1978) was used as a test for antidepressant activity. The mice were randomly divided into 5 groups of 6 animals each, which were individually forced to swim for 10 min, 24 h before the first treatment, in an open glass cylinder (h=48 cm, d=18 cm) containing fresh tap water up to a height of 25 cm at 24±1 °C. Each group was receiving for 10 consecutive days s.c. injection (2.5 ml/kg) of either imipramine: 10 mg/kg, herbal extract: 2, 6 or 20 mg/kg (where gentiacaulein participated by its calculated concentration: 5.3, 15.8 and 52.8 μM/kg, respectively), or 4% DMSO dissolved in physiological saline. On the first and the last day of the treatment, 60 min after the injection, each single animal was placed into the water and, from the second minute onward, its immobility was rated during 5-min period at 5-sec intervals. The animal that kept floating, with only essential movements to keep its head above the water, was considered immobile. The mean immobility score for each experimental group was calculated.

2.4.2. Open-field test

The motor activity of the animals was monitored in the open-field by an automatic device (Columbus Auto-Track System, Version 3.0 A, Columbus Institute, OH, U.S.A.). Each monitoring instrument (Opto-Varimex) consisted of a Plexiglas cage (44.2×43.2×20 cm) connected to the Auto-Track interface. It was intersected by the grid of horizontal and 2-level vertical infrared beams, where the distance between adjacent beams was 2.4 cm. Interruption of a beam generated an electrical impulse, which was subsequently processed and sent to the computer linked to Auto-Track interface. To eliminate any interaction of animals with the environment during the experimental sessions, Opto-Varimex devices were placed into the light- and sound-attenuated chambers, with artificially regulated ventilation and illumination (100 l×).

The type of activity, characterized by the animal movements, was determined by the user-defined box size (set to 2 beams for mice). The described parameters were defined in accordance with Auto-Track system for IBM-PC/XT/AT version 3.0A (Instruction Manual 0113-005L, 1990), providing the data on ambulatory and stereotypic activity. The mouse had to break more than 2 horizontal infrared beams for the activity to be classified as an ambulatory.

Their locomotion was presented by the distance traveled (in cm) and by the total ambulatory time, for which the animal had passed this distance. Stationary activity, registered by the actual number of beams that were broken inside the space that covered the dimension of an animal (defined by the outer borders of the cube formed by 2 adjacent horizontal and 2 vertical intercrossing beams), was implied as a stereotypic movement. Stereotypy was presented by bursts of stereotypic movements, which was mostly consisted of repetitive sniffing and grooming, and by vertical counts, which generally represented rearing of an animal. Stereotypic time was the period spent in stereotypic behavior. Resting time was presented as the period without ambulatory or stereotypic movements. The treatment regimen, separation in groups and number of animals in open-field test was the same as for the FST. Each mouse was experimentally naive and tested only once on the 10th day of treatment. Animals were placed for 15-min adaptation in the center of experimental box, 45 min after the last injection. Automatic recording of the behavioral parameters was performed during the next 15 min.

2.5. Ex vivo MAO assays

All mice used in behavioral experiments were sacrificed immediately after the tests (forced swimming or open-field), i.e. about 90 min after the last injection of the 10-day treatment. The brains excised and frozen rapidly were kept at −70 °C for up to 3 weeks, until MAO assays. Individual brains were thawed and homogenized (a Teflon-glass homogenizer, 8 strokes, 1000 rpm, 4 °C) in 20 vol. 0.3 M sucrose solution in 20 mM K-phosphate buffer, pH 7.4. The homogenates were centrifuged (1500 × *g*, 5 min, 4 °C) and the supernatants collected. Crude mitochondrial pellets obtained by centrifugation (15 000 × *g*, 30 min, 4 °C) of the supernatants were resuspended in 4 ml of 50 mM K-phosphate buffer, pH 7.5 and used for MAO assays. The non-sedimented fractions remained after centrifugation, containing free enzymes occurring outside synaptosomes, were also analyzed for MAO activity. Ex vivo MAO activities were determined radiometrically, analogously to the method used for in vitro MAO assays described above. They were calculated in relation to the protein concentration in the samples (Markwell et al., 1978). MAO activity was expressed as a percentage of vehicle-treated control ± S.E.M.

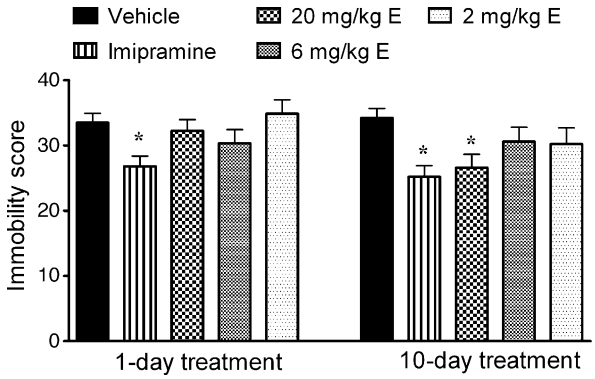

Fig. 2. Effects of a single and 10-day treatment (s.c.) with 10 mg/kg b.w. of imipramine and three doses (20, 6 and 2 mg/kg b.w.) of diethylether extract of *G. kochiana* on immobility score in forced swimming test. Each column represents the mean ± S.E.M. for $n = 6$ animals. *Significant difference from control (vehicle, 4% DMSO in 0.9% NaCl) at $p < 0.05$ (Dunnett's post ANOVA test).

2.6. Statistical analysis

The analyses of the data from in vitro and ex vivo assays and testing of behavioral inter-group differences were performed by GraphPadPrism 4.0 for Windows. The IC_{50} values (concentration of compounds that induced 50% inhibition of the examined processes or receptor binding) were calculated by nonlinear regression curve fit with one-site competition. One-way ANOVA was used for statistical analysis of behavioral parameters. It was followed by Dunnett's post hoc test for multiple comparisons if overall differences were significant ($p < 0.05$). Bartlett's test was employed to pre-test the equality of variances.

3. Results

3.1. In vitro assays

The results obtained by pharmacological in vitro assays (Table 1) showed that neither the Et_2O extract, nor the xanthones expressed a significant interaction with either DA or 5-HT receptors. Also, the explored compounds did not influence synaptosomal 5-HT uptake. However, the inhibitory potency of the extract and the two xanthones on MAO enzymes was confirmed. Gentiacaulein exhibited a

Table 1
In vitro effects of diethylether (Et_2O) extract and xanthones of *G. kochiana* on dopamine (DA) and serotonin (5-HT) receptors, 5-HT reuptake and monoamino oxidase (MAO) activity

Compounds	IC_{50}							
	D_1	D_2	5-HT_{1A}	5-HT_{2A}	5-HT_{2C}	5-HT uptake	MAO A	MAO B
Et_2O extract (μg/ml)	NS[a]	NS	NS	NS	NS	NS	0.22 ± 0.05	96.0 ± 18.6
Gentiacaulein (μM)	NS	NS	NS	NS	NS	NS	0.49 ± 0.12	340 ± 102
Gentiakochianin (μM)	NS	NS	NS	NS	NS	NS	164 ± 20	63.0 ± 9.4

Values are expressed as mean ± S.E.M., $n = 4$.
[a] NS: not significant, without registered inhibition or $IC_{50} \gg 1.0$ mg/ml (extract) or 1.0 mM (xanthones).

rather strong MAO blocking potential, predominantly as MAO A inhibitor (IC_{50}=0.49 μM). The same holds true for the action of the extract (IC_{50}=0.22 μg/mg), containing a high concentration of gentiacaulein. Gentiakochianin acted as much weaker MAO A inhibitor (IC_{50}=164 μM) and both xanthones expressed a moderate MAO B inhibition.

3.2. Forced swimming test

Fig. 2 shows the effects of a single (left) and 10-day administration (right) of imipramine (10 mg/kg) and three different doses of *G. kochiana* Et_2O extract (2, 6 or 20 mg/kg) on the immobility score in adult mice males. One-way ANOVA analysis pointed to significant differences in immobility score among experimental groups after the first treatment [$F(4,25)=3.004$, $p=0.0374$], and, also, following the repeated treatment [$F(4,25)=3.079$, $p=0.0343$]. Post-test demonstrated that only imipramine substantively decreased the immobility score after both, 1-day and 10-day administration (−20%, $p<0.05$ and −27.3%, $p<0.05$, respectively). However, among the animals treated with the extract, a significant decline of the measured parameter was observed only in the group receiving 20 mg/kg of the extract for 10 days (−22.2%, $p<0.05$). Subacute administration of the extract in two lower doses also induced some 10–12% reduction of immobility scores that appeared to be non-significant.

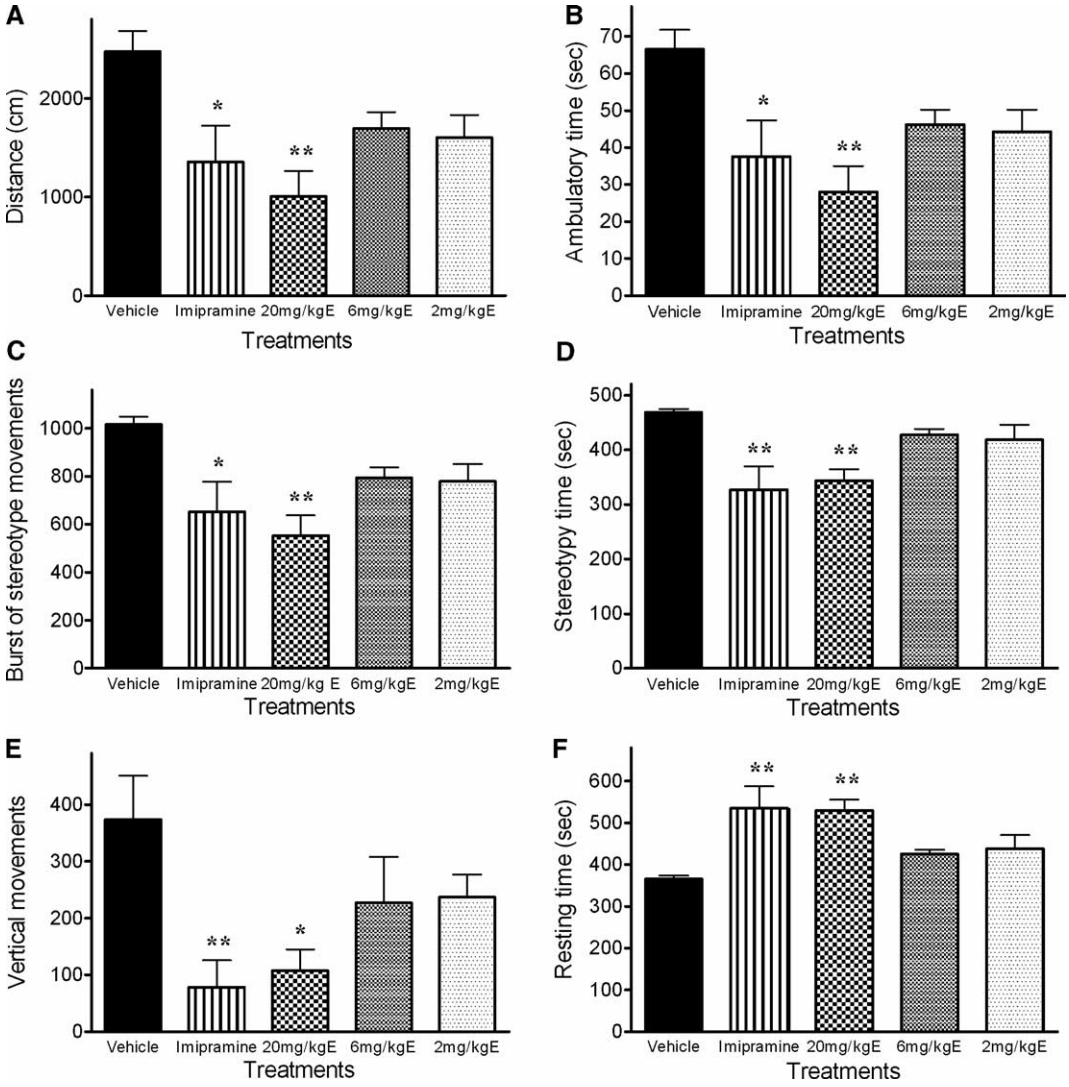

Fig. 3. Effects of 10-day treatment (s.c.) with imipramine (10 mg/kg b.w.) and diethylether extract of *G. kochiana* (20, 6 and 2 mg/kg b.w.) on open-field behavioral parameters. The parameters were automatically recorded by Columbus Auto-Track System, during a 15-min-period, starting 60 min after the last injection. Ambulation was registered by the distance traveled (A), total stereotypy—by burst of stereotypic movements (C) and rearing—by vertical movements (E). The full time was recorded as the ambulatory time (B), the time for stereotypic movements (D) and the resting time (F). The bars represent mean values ± S.E.M. for groups consisting of 6 animals each. *Significant difference from control group (vehicle) at $p<0.05$, **$p<0.01$ (Dunnett's post ANOVA test).

3.3. Open-field behavior

All parameters of an open-field analysis suggested that 10-day treatment with imipramine or Et_2O extract of *G. kochiana* may induce locomotor reduction and decreased stereotype behavior of mice (Fig. 3 A–F). ANOVA test confirmed a significant influence of the treatments on the distance traveled [$F(4,25)=4.542$, $p=0.0068$], and ambulatory time [$F(4,25)=4.449$, $p=0.0075$]. Also, there were significant changes of stereotypy time [$F(4,25)=5.58$, $p=0.0024$], burst of stereotypic movements [$F(4,25)=4.965$, $p=0.0044$] and vertical movements [$F(4,25)=3.911$, $p=0.0134$]. Dunnett's post-test revealed a significant decrease of the mentioned parameters (up to 80%) only in the groups receiving imipramine and 20 mg/kg of the extract. On the other hand, these two groups exhibited an increased resting time [+45–46%; $F(4,25)=5.519$, $p=0.0025$].

3.4. Ex vivo MAO activity

The influence of subacute treatments of mice with Et_2O extract of *G. kochiana* and imipramine on the ex vivo deaminating activity of the brain mitochondrial MAO enzymes (Fig. 4 A and B4), appeared to be significant only for MAO A [$F(4,55)=3.152$, $p=0.021$], and not for MAO B [$F(4,55)=1.333$, $p=0.2694$]. Dunnett's post test showed an unexpected significant increase of MAO A activity (20.3%, $p<0.01$) in the group of animals receiving 20 mg/kg of the extract. The residual MAO A activity outside nerve terminals (Fig. 4 C), determined in the suspensions of proteins that remained after preparation of crude mitochondrial fractions, was generally decreased in all groups [$F(4,55)=3.120$, $p=0.022$]. However, this decrease was significant only in the group of animals administered 20 mg/kg or 6 mg/kg of the extract (−22.7%, $p<0.01$, and −18.1%, $p<0.05$, respectively). No similar changes of MAO B activity were observed in either of the treated groups [$F(4,55)=0.404$, $p=0.805$].

4. Discussion

The results of the present study clearly demonstrated the effects of the active compounds of *G. kochiana* on neurochemical processes and behavior in rodents. *Via* screening the effects of methanol, Et_2O, ethyl acetate and *n*-butanol extracts of *G. kochiana* aerial parts on in vitro MAO enzyme activity, we have observed previously that Et_2O extract expressed the highest MAO blocking potential (Tovilović et al., 2004). Additionally, HPLC analysis showed that this Et_2O extract is composed mostly of two xanthones (>90%), gentiacaulein and gentiakochianin. These purified xanthones and the Et_2O extract itself were

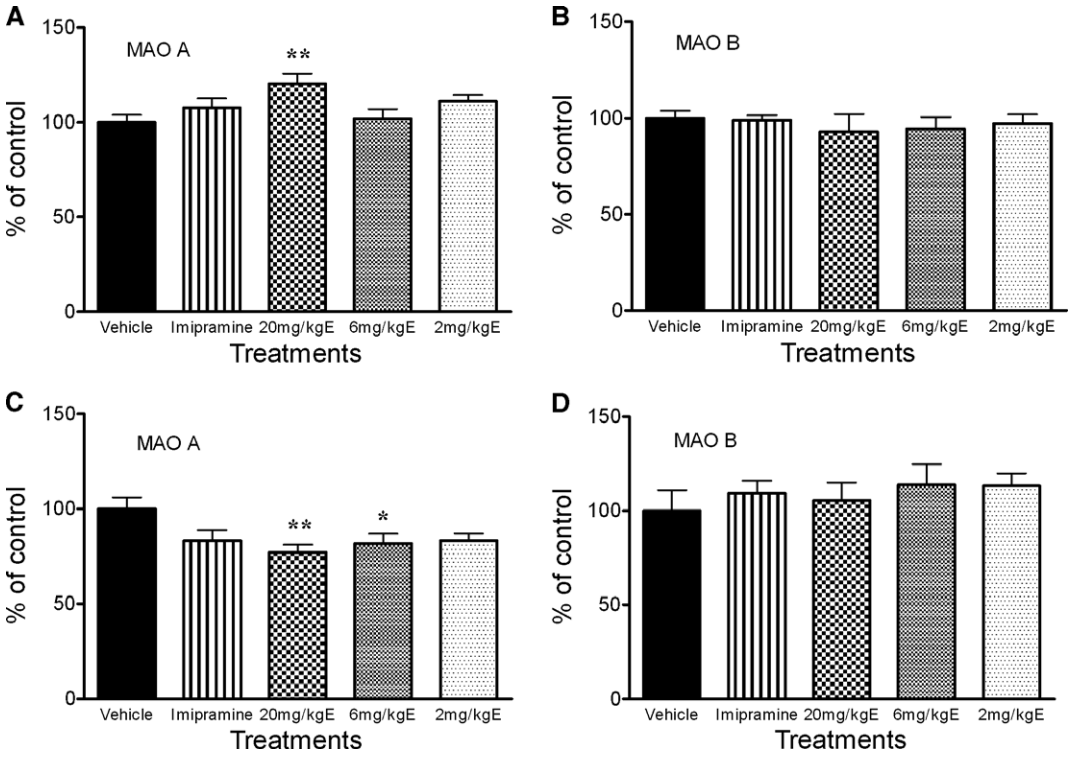

Fig. 4. Effects of 10-day treatment with imipramine (10 mg/kg, s.c.) and diethylether extract of *G. kochiana* (20, 6 and 2 mg/kg b.w.) on murine brain MAO A and MAO B. Ex vivo MAO activity of crude mitochondrial preparations (A, B) and of supernatants remained after centrifugation (C, D) are presented as percentage (%) of control values. The bars represent mean values±S.E.M. for groups consisting of 12 animals each. *Significant difference from control group (vehicle) at $p<0.05$, **$p<0.01$ (Dunnett's post ANOVA test).

used for the more comprehensive in vitro pharmacological analyses. Neither of the compounds examined in the present work showed any notable interaction on either DA and 5-HT receptor binding or 5-HT uptake. However, an inhibitory potency of the tested compounds on MAO enzymes was confirmed. This is in agreement with the results of other in vitro studies, which proved that some natural xanthones or their derivatives inhibited MAO enzymes in a competitive manner (Suzuki et al., 1980, 1981; Schaufelberger and Hostettmann, 1984; Thull and Testa, 1994; Ohishi et al., 2000). As early as in 1981, Suzuki and coworkers suggested that naturally occurring gentiacaulein acts as a strong MAO A inhibitor in vitro, although without determining the exact IC_{50} value. Our experimental data clearly demonstrate substantial MAO A inhibiting activity of gentiacaulein ($IC_{50}<1.0$ μM) and a similar inhibitory potency of the Et_2O extract. The effect of the extract should depend entirely on gentiacaulein as a dominant component (76%), acting as conspicuously stronger MAO A inhibitor than gentiakochianin, while the latter appeared to be slightly more potent MAO B inhibitor than the former. Transformation of the experimental IC_{50} value of the extract for MAO A inhibition (0.22 μg/ml) to a participated concentration of gentiacaulein (0.58 μM), showed that it is comparable, though somewhat higher than IC_{50} determined directly for gentiacaulein by the assay (0.49 μM). This calculation excludes any synergism of gentiacaulein with gentiakochianin, or any other minor component contained in the Et_2O extract, regarding in vitro MAO A inhibition.

Behavioral studies on mice demonstrated that Et_2O extract of *G. kochiana*, when applied subcutaneously in a repetitive manner, affected both FST and open-field behavior with the effects resembling those induced by similar imipramine treatment. Only the highest dose of the extract (20 mg/kg) was active in behavioral tests. A clear dose response relation was difficult to determine due to the limited solubility of the extract. It seems that the antidepressant potential of the extract, suggested by FST, is a result of MAO A inhibition induced mostly by gentiacaulein, while the mechanism of ambulatory and stereotypy reduction following subacute treatment with the extract or imipramine is still controversial. Some authors reported that repetitive imipramine treatment either stimulates or has no effect on rat locomotion (Meltzer and Fox, 1971; Mogensen et al., 1994). Nevertheless, some other studies showed that imipramine, as well as a few other antidepressants, might induce somewhat depletion of locomotor behavior of rodents and this was only partially explained by the activation of brain serotonin system (Kameyama et al., 1985; Wenzel and Kuschinsky, 1990; Brocco et al., 2002). Even so, it is uncertain if the predictable in vivo MAO A blockade induced by the *G. kochiana* extract would be sufficiently strong to provoke behavioral effects registered throughout the present study. This question is motivated by ex vivo evaluation of mitochondrial MAO A originating from the brains of mice subacutely treated with 20 mg/kg of the extract, which revealed an increased activity of the enzyme, although a free non-mitochondrial MAO A activity appeared to decline following both 6 and 20 mg/kg 10-day treatment. Since it is regarded that the free portion of brain MAO A does not play the principal role in monoamino degradation, these findings should be in collision with an assumption that the xanthone-induced MAO A inhibition represents the basis for the registered behavioral effects of the extract. However, taking into account the fact that simple oxygenated xanthones act as reversible MAO inhibitors (Suzuki et al., 1981; Schaufelberger and Hostettmann, 1984; Peres et al., 2000), while their concentration in samples may considerably decrease during ex vivo brain preparation, it is likely that the results obtained ex vivo may conceal a probable in vivo inhibition of synaptosomal MAO A. It is not apparent if such inhibition, generated by gentiacaulein during the repeated application of the *G. kochiana* extract, could induce de novo synthesis of mitochondrial MAO A. This route may be taken as a possible explanation of the elevated ex vivo mitochondrial MAO A activity registered in the present study, although the processes of MAO synthesis were not the subject of this research. Nevertheless, the reports suggesting the stimulation of de novo MAO production were usually based on experiments with irreversible MAO inhibitors (Felner and Waldmeier, 1979; Fagervall and Ross, 1986), while the recovery of MAO activity upon reversible in vivo inhibition should be only spontaneous one (Blier et al., 1997).

Taking all together, the present findings suggest some therapeutic antidepressant potential of *G. kochiana,* with a probable additional sedative effect. These outcomes are presumably connected to the action of gentiacaulein, while the complete pharmacological mechanism is not entirely clear. Muruganandam et al. (2000) suggested an important contribution of trioxygenated xanthones to the antidepressant activity of *Hypericum perforatum* by the mechanism of DA D_2 receptors downregulation and upregulation of 5-HT_2A receptors. The in vitro tests performed throughout the present study were also stimulated by the acknowledged viewpoint that brain 5-HT and DA systems play a key role in the regulation of mood and motor behavior, but, apart from MAO inhibition, they did not indicate any specific influence of the researched compounds on some important elements of these systems. However, there are some other systems of neurotransmission and neuromodulation (e.g. GABA, noradrenaline, neuropeptidergic) that may influence the observed behavioral parameters. Based on behavioral effects of the chiral xanthone derivatives, such as the reduction of total locomotor activity, immobility time in FST and anticonvulsant activity in picrotoxin-induced seizures, Jastrzebska-Wiesek et al. (2003) suggested a possible behavioral impact of xanthones through their interaction with GABA system. So, additional evaluation

of the mechanisms underlying pharmacological actions of xanthones of *G. kochiana* in the CNS would be justified. Further studies along this line are in progress.

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