ORIGINAL ARTICLE

EFFECTS OF AQUEOUS INFUSION FROM *COTINUS COGGYGRIA* LEAVES ON BEHAVIOR AND LIPID PEROXIDATION IN RATS

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Abstract

The aim of this study was to investigate the effects of an aqueous infusion from *Cotinus coggygria* leaves (AICCL) on behaviour and on lipid peroxidation in male Wistar rats. The animals were treated orally with three concentrations (1/100, 2/100 and 4/100) AICCL at doses of 10 mL/kg b.w. Control rats received distilled water (10 mL/kg b.w.). After 30 days of treatment, the open field test (OFT) and the forced swim test (FST) were carried out and the lipid peroxidation marker, malondialdehyde (MDA) was measured as a biomarker of oxidative stress. In the OFT, AICCL at all concentrations did not significantly affect the horizontal activity and dose-dependently increased the vertical activity, the effect being significant ($p < 0.05$) at the highest concentration. In the FST, AICCL shortened the immobility time and the effect was significant ($p < 0.05$) at the concentration of 2/100. AICCL did not cause sedation and motor discoordination, and reduced the signs of depression. AICCL caused a tendency to decrease MDA levels in both serum and brain homogenate, but the reduction was not statistically significant. Thus, the favourable effects of AICCL on brain functions in this experiment cannot be attributed to the antioxidant action.

Rezumat

Acest studiu a avut ca scop evaluarea posibilelor efecte infuziei apoase din frunze de *Cotinus coggygria* (*AICCL*) asupra comportamentului și a peroxidării lipidice la șobolani Wistar de sex masculin. Animalele au primit *per os* trei concentrații de (1/100, 2/00 și 4/100) *AICCL*, în doze de 10 mL/kg corp. Lotul control a primit apă distilată 10 mL/kg corp. După 30 de zile de tratament, au fost determinate testul câmpului deschis (*OFT*) și testul înotului forțat (*FST*), malondialdehida (MDA) peroxidării lipidice a fost determinată pentru toate concentrațiile ca un biomarker al stresului oxidativ. În *OFT*, *AICCL* nu a afectat semnificativ activitatea iar în funcție de doză a crescut activitatea verticală, efectul fiind semnificativ ($p < 0,5$) pentru cea mai mare concentrație. În *FST*, *AICCL* a diminuat timpul de imobilizare, și efectul a fost semnificativ ($p < 0,5$) pemtru concentrația de 2/100. *AICCL* nu a provocat sedare sau lipsă de coordonare motorie și a redus semnele de depresie. *AICCL* a scăzut ușor nivelul MDA atât seric cât și cerebral dar scăderea nu a fost semnificativă statistic. Astfel, efectele favorabile ale *AICCL* asupra funcțiilor cerebrale, în experiment nu poate fi atribuită acțiunii antioxidante.

Keywords: *Cotinus coggygria*, locomotor activity, anti-depressant effect, rats

Introduction

The Eurasian Smoke tree *(Cotinus coggygria* Scop., *Anacardiaceae)* is a well-known medicinal plant on the Balkan Peninsula. In folk medicine, the infusions from the leaves are applied topically for their antiseptic, anti-inflammatory, antimicrobial, anti-haemorrhagic and wound-healing properties. Because of the high gallotannin content (above 25%), some authors [7, 20] consider the whole plant poisonous and it is seldom used orally. However, there are few reports on oral use of infusions from the leaves for the treatment of throat and stomach inflammation, gastric ulcers, diarrhoea and even diabetes mellitus [2, 6, 10]. The chemical substances present in the leaves infusion are gallic acid methyl ester and anthocyanins [12], galotanins, gallic acid, flavonic glycosides, myrcen, alpha-pinene, camphen, linalool, and alpha-terpineol [10, 21]. Numerous polyphenolic compounds have been isolated, including quercetin, fustin, and taxifolin [19]. ^{14}C-labeled plant polyphenols found in the brain tissue and brain microdialysate indicate that these phytochemicals or their metabolites are able to cross the blood-brain barrier [5]. Although the plant seems to be extremely rich in biologically active compounds, it has been somewhat ignored by pharmacological studies because of reported toxicity. Our previous studies showed that the subchronic administration of aqueous infusion from *C. coggygria* leaves at concentrations of 1/100, 2/100 and 4/100 did not cause toxicity to the liver and kidneys [13].

The aim of this study was to investigate the toxicity of an aqueous infusion from *C. coggygria* leaves (AICCL) at the brain level in Wistar rats using behavioral tests and the marker of lipid per-oxidation malonedialdehyde (MDA) as a measure of oxidative stress.

Materials and Methods

Experimental substances

All infusions were obtained from identical and standardized plant material that was purchased from Bilek Ltd., Troyan, Bulgaria. Three concentrations (1/100, 2/100 and 4/100) of aqueous infusions from *Cotinus coggygria* leaves (AICCL) were prepared one hour before each treatment as 1 g, 2 g and 4 g of dried material were infused in 100 mL of boiling distilled water for 10 min. The infusions were filtered through a cotton lint.

Animals

Male Wistar rats (200 ± 20 g) were used. The animals were housed in plastic cages in a well ventilated room maintained at 22 ± 1°C and on a 12/12 light/dark cycle. They had free access to food and drinking water. All procedures concerning animal treatment and experiments were conducted in compliance with National and International laws and policies (EEC Council Directive 86/609).

Experimental protocol

The rats were divided into 4 groups of 10 animals each: control, AICCL1, AICCL2, AICCL4. They were treated once daily, orally through an orogastric cannula for a period of 30 days. Control rats received distilled water (10 mL/kg b.w.). Animals from AICCL1, AICCL2 and AICCL4 groups were respectively treated with 1/100, 2/100 and 4/100 AICCL aqueous infusions (at a dose 10 mL/kg b.w.). After the experimental period, the open field test (OFT) and the forced swim test (FST) were carried out.

Open field test (OFT)

OFT is a common measure of exploratory behaviour and general activity in rodents [3]. It was performed for 5 min in an arena (100 × 100 × 40 cm) painted white except for 6 mm blue lines that divided the floor into 25 equal size (20 × 20 cm) squares. Each animal was placed in the centre of the uniformly lit arena and its behaviour was observed in silence for 5 min. Behaviours recorded were: crossings (the number of lines crossed with the four paws) and rearings (the number of times the animal stood on its hind limbs).

Forced swim test (FST)

The method of Porsolt *et al.* [14] was used to assess the immobility of the rats as a measure of their depression-like behaviour. Each rat was placed in a glass cylinder pool (17 cm in diameter and 60 cm in height) for 5 min. The cylinder was filled with 30 cm water (21 ± 1°C) to ensure that the animal could not touch the bottom of the cylinder with its hind paws or its tail. The test was performed in two sessions with a 24 h interval. The results from the second session were recorded. Inactivity (immobility) and swimming were distinguished as mutually exclusive behavioural states. Swimming behaviour was defined as movement throughout the cylinder. Immobility was defined when no additional activity was observed other than that required to keep the rat's head above the water. The increased immobility time is a measure of depression-like behaviour.

Malondialdehyde measurement

Membrane lipid peroxidation was monitored by malondialdehyde (MDA) in rat serum and brain homogenate according to Porter *et al.* [15]. The method is based on the ability of MDA in acidic medium (pH about 3) and high temperature (96ºC) to form with thiobarbituric acid chromophores (TBC), having a maximum absorption at 532 nm.

Statistical analysis

Results are presented as mean ± S.E.M. The data were tested by one-way ANOVA, followed by Dunnett's multiple comparison post-test to identify significant difference. All analyses were performed using GraphPad Prism statistical software. A level of $p < 0.05$ was considered significant.

Results and Discussion

Open field test

The results from the OFT, showed that the treatment with AICCL did not significantly increase the horizontal locomotor activity of the rats belonging to groups AICCL1 (58.1±11.1), AICCL2 (46.9 ± 4.9) and AICCL4 (47.1 ± 5.4) compared with the control group (39.4 ± 6.3). The vertical activity has dose-dependently increased from 16.0 ± 2.8 for the control group, to 18.6 ± 2.2 for AICCL1, 20.6 ± 1.6 for AICCL2 and 24.9 ± 3.0 for AICCL4 ($p < 0.05$ *vs.* control) (Figure 1).

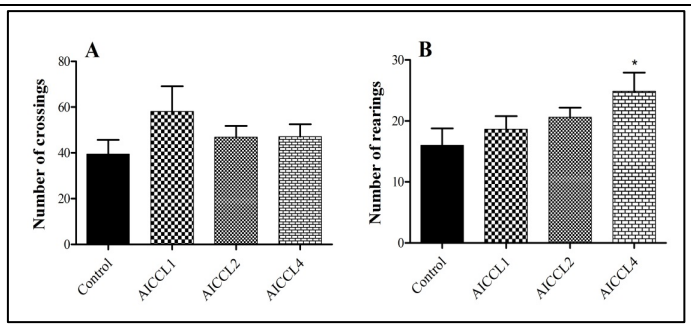

Figure 1.

Effect of aqueous infusion from *Cotinus coggygria* leaves (AICCL) administered for 30 days at concentrations of 1/100, 2/100 and 4/100 on the horizontal (panel A) and vertical (panel B) locomotor activity of rats; *p < 0.05 *vs.* control

Forced swim test

In the FST, the immobility time of the control animals was 89.0±8.0 sec. It was shortened in AICCL-treated rats to 66.2 ± 5.6 sec for AICCL1, 55.3 ± 8.4 sec (p < 0.05 *vs.* control) for AICCL2 and 67.9 ± 11.7 sec for AICCL4 (Figure 2).

Malondialdehyde assay

Malondialdehyde levels of the controls were 1.33 ± 0.06 µmol/L in blood serum (Figure 3A) and 9.81 ± 1.30 nmol/g in brain homogenate (Figure 3B). The aqueous infusions from *Cotinus coggygria* leaves caused a tendency to decrease MDA levels in both blood serum (Figure 3A) and brain homogenate (Figure 3B) but the reduction was not statistically significant.

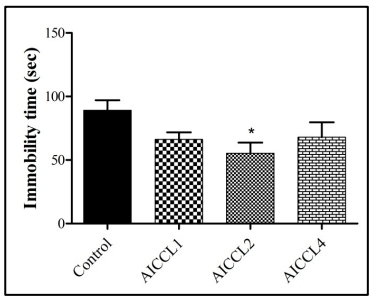

Figure 2.

Effect of aqueous infusions from *Cotinus coggygria* leaves (AICCL) administered to rats for 30 days at concentrations of 1/100, 2/100 and 4/100 on the immobility time in the FST; *p < 0.05 *vs.* control

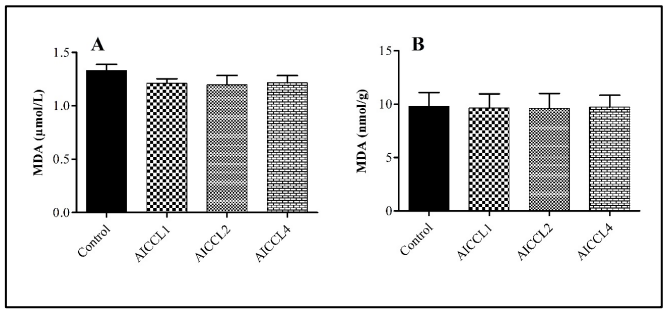

Figure 3.

Effect of aqueous infusions from *Cotinus coggygria* leaves (AICCL) administered for 30 days at concentrations of 1/100, 2/100 and 4/100 on the malondialdehyde (MDA) levels in blood serum (panel A) and brain (panel B) of rats

Our previous data [13] showed that AICCL at concentrations of 1/100, 2/100 and 4/100 administered to rats for 30 days did not cause liver toxicity proven by the lack of effect on serum concentrations of the liver enzymes aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase, as well as by histo-pathological examination of the liver. These concentrations of AICCL were also not toxic for the kidneys as they did not significantly affect serum concentrations of creatinine and urea in the treated animals [13].

In the present study, the brain toxicity of sub-chronic administration of AICCL at concentrations of 1/100, 2/100 and 4/100 was investigated. The OFT showed that AICCL did not affect adversely the horizontal and vertical locomotor activity in rats. On the contrary, there was a significant increase in the vertical activity of the animals treated with 4/100 AICCL. Based on these results we could conclude that the sub-chronic

administration of AICCL did not cause sedation and motor discoordination, therefore did not exhibit toxic effects on the central nervous system of rats. The FST demonstrated that the sub-chronic administration of AICCL decreased the immobility time which indicated that *C. coggygria* might have an anti-depressant activity.

The brain is very vulnerable to oxidative stress. The major depressive disorders are associated with reduced levels of certain endogenous antioxidants and a disturbance in the total antioxidant status. These observations bring new opportunities for therapeutic interventions based on antioxidant substances [17].

The effects of AICCL in the behavioural tests could be attributed to its polyphenolic ingredients which are the main biologically active substances in *C. coggygria* leaves according to literature data [10, 12, 19, 21].

There are data that *C. coggygria* infusions possess a high antioxidant activity *in vitro* [4, 9]. The results of the present experiment showed that AICCL caused an insignificant decrease in lipid peroxidation measured by serum and brain MDA concentrations. Thus, the favourable effects of AICCL on brain functions in this experiment might not be attributed to its antioxidant action.

There are data considering the role of inflammation in the pathogenesis of depression. Pro-inflammatory cytokines such as IL-6 and TNF-α are the most reliable markers for inflammation in patients with this disease [1]. The pro-inflammatory cytokines interact with mitochondria and increase the production of reactive oxygen species (ROS) which in turn increase the expression of cytokines [18].

Noradrenaline and 5-hydroxytriptamine (5-HT) are the main neurotransmitters the depletion of which leads to depression. Previous studies have shown that green tea polyphenols inhibit monoamine oxidase (MAO) and thereby increase the level of monoamines in the glial cells [8]. There are similar data for *Mentha aquatica* which contains the flavonoid naringenin [11]. The rich in catechins, procyanidins and flavonoids *Cecropia glazioui* Sneth extract and six of its purified constituents possess an antidepressant-like effect most likely due to the blockade of the uptake of monoamines (5-HT, dopamine and noradrenaline) by synaptosomes in different brain regions [16].

Thus, the anti-depressant effect of AICCL could be the result of anti-inflammatory and monoamine-increasing effects. This assumption has already been supported by some observations. *C. coggygria* infusions have been traditionally used for their anti-inflammatory properties. The anti-inflammatory effect of AICCL has also been demonstrated in our experiments in a model of carrageenan-induced rat paw oedema (unpublished data).

Conclusions

The effects of AICCL in the behavioral test in rats were studied as a part of the subchronic toxicity study of the infusion. AICCL had no adverse effects on the locomotor activity and possessed an anti-depressant effect. The results showed that AICCL administered subchronically to rats for a period of 30 days was not toxic for the central nervous system and could be further investigated for possible beneficial effects in animal models of different diseases.

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