



## Anxiolytic and antidepressant effect of zinc on rats and its impact on general behavioural parameters

Anksiolitički i antidepressivni efekat cinka na pacove i njegov uticaj na opšte bihevioralne parametre

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### Abstract

**Background/Aim.** Zinc is an essential element which has considerable interaction with gamma-aminobutyric acid A type receptors (GABA<sub>A</sub>) and glutamate receptors in the central nervous system (CNS). It is believed that zinc acts as a potent inhibitor of glutamate N-methyl-D-aspartate (NMDA) receptors, and binding to structurally specific site on the GABA<sub>A</sub> receptor leads to inhibition of GABA-dependent Cl<sup>-</sup> pass. The aim of our research was to test the anxiolytic and antidepressant effects of zinc after single application and its influence on general behavioural parameters after repeated administration. **Methods.** Male Wistar rats were treated with increasing doses of zinc histidine dehydrate (10, 20, 30 mg/kg, i.p.). To determine anxiolytic and antidepressant properties of zinc two models were used: elevated plus maze (EPM) and forced swim test (FST). Behavioural parameters (stillness and mobility) were, also, recorded after single and repeated administration of active substance. **Results.** Testing animals in the EPM showed a statistically significant differ-

ence as follows: dose of 20 mg/kg significantly increased the time animals spent in open arms, indicating an acute anxiolytic effect, while doses of 30 mg/kg significantly reduced the time in the open arms, indicating a potentially anxiogenic effect. Testing the animals by FST showed a statistically significant difference in immobility time of animals treated with the lowest applied (10 mg/kg) and highest applied (30 mg/kg) doses of zinc, compared to the control group. The first day of testing behavioral parameters showed the tendency to increase locomotor activity of the animals with the lowest dose of zinc (10 mg/kg), while the following day revealed a reduced activity with the highest dose applied (30 mg/kg). **Conclusion.** Zinc has important effects on the CNS: After single application, in all doses zinc showed antidepressant effects. The effects of zinc on anxiety and locomotor activity showed dose-dependent bidirectional effects.

**Key words:** zinc; rats; anti-anxiety agents; antidepressive agents, second generation.

### Apstrakt

**Uvod/Cilj.** Cink je esencijalni element, koji u centralnom nervnom sistemu (CNS) ostvaruje značajnu interakciju sa tipom A receptora za gama aminobuternu kiselinu (GABA<sub>A</sub>) i glutamatskim receptorima. Smatra se da cink deluje kao snažan inhibitor glutamatskih N-metil-D-aspartat (NMDA) receptora, a vezivanjem za strukturno specifično mesto na GABA<sub>A</sub> receptoru dovodi do inhibicije GABA-zavisne Cl<sup>-</sup> struje. Cilj našeg istraživanja bio je da ispitamo anksiolitičke i antidepressivne efekte cinka posle jednokratne primene i njegov uticaj na opšte bihevioralne parametre

posle ponavljanoj davanju. **Metode.** Mužjaci pacova soja Wistar tretirani su rastućim dozama cink-histidin dehidrata (10, 20, 30 mg/kg, *ip*). Za ispitivanje anksiolitičkih i antidepressivnih svojstava cinka korišćena su dva testa: uzdignuti plus lavirant (EPM) i test forsiranog plivanja (FST). Praćeni su, takođe, bihevioralni parametri (mirovanje i aktivnost životinje) tokom jednokratne i ponavljane primene aktivne supstance. **Rezultati.** Testiranjem životinja primenom EPM utvrđena je statistički značajna razlika: životinje koje su primile dozu od 20 mg/kg provodile su statistički značajno više vremena u otvorenim kracima, što ukazuje na akutni anksiolitički efekat, dok je doza od 30 mg/kg

značajno skraćivala vreme koje životinje provode u otvorenom prostoru lavirinta. Ovo ukazuje na potencijalno anksiogene efekte cinka. Testiranjem životinja primenom FST dokazana je statistički značajna razlika u vremenu imobilnosti životinja tretiranih najmanjom primenjenom dozom (10 mg/kg) i najvećom primenjenom dozom cinka (30 mg/kg), u odnosu na kontrolnu grupu. Prvog dana ispitivanja bihevioralnih parametara pokazana je tendencija povećanja lokomotorne aktivnosti životinja sa najmanjom primenjenom dozom cinka (10 mg/kg), dok je narednog

dana uočena tendencija snižavanja aktivnosti sa najvećom primenjenom dozom (30 mg/kg). **Zaključak.** Cink ispoljava značajne efekte na CNS. Jednokratna primena, cinka, u svim dozama pokazuje antidepresivne efekte. Efekti cinka na anksioznost i lokomotornu aktivnost pokazuju dozozavisne dvosmerne efekte.

**Ključne reči:**

**cink; pacovi; anksiolitici; antidepresivi druge generacije.**

## Introduction

Zinc is an essential element, important for the function of over 200 enzymes. The role of zinc in humans is catalytic, structural and cofactorial, and is required for DNA replication, transcription and protein synthesis<sup>1, 2</sup>. In the central nervous system (CNS), the presence of zinc has been confirmed in the neocortex, amygdala and hippocampal structures. In Zn-containing neurons, zinc is stored in presynaptic vesicles and the vesicles are then released according to the depolarisation and the presence of calcium<sup>3</sup>. Zinc has considerable interaction with gamma-aminobutyric acid A type receptors (GABA<sub>A</sub>) and glutamate receptors, as well as with voltage-dependent sodium, potassium and calcium channels<sup>4</sup>.

The mechanism of zinc action on the CNS, to date, has not been fully determined<sup>5</sup>. Zinc, in the CNS, binds to glutamate N-methyl-D-aspartate (NMDA) receptors and acts as a potent modulator of glutamate neurotransmission<sup>3, 6</sup>. It is known that zinc binds to a structurally specific binding site on the GABA<sub>A</sub> receptor, and may lead to inhibition of GABA-dependent Cl<sup>-</sup> ions passage<sup>7</sup>. It is shown that the sensitivity of different types of GABA<sub>A</sub> receptors to the effects of zinc is different and that depends on the structural subunits of GABA<sub>A</sub> receptor complex<sup>7, 8</sup>.

Numerous studies suggest the important role of this essential element in pathogenesis of neuropsychiatric disorders, such as epilepsy<sup>9, 10</sup>, mood disorders<sup>5, 11, 12</sup> and neurodegenerative diseases<sup>13, 14</sup>. In preclinical models, which are used for the evaluation of antidepressant activity, zinc shows effects similar to antidepressants<sup>5, 15, 16</sup>. It has been found that chronic use of antidepressant drugs, such as citalopram or imipramine and electroconvulsive therapy, increases the concentration of zinc in the hippocampus of rats<sup>5, 17</sup>. It has also been shown that chronic use of citalopram increases the concentration of zinc in serum, while imipramine and electroconvulsive therapy have not shown such an effect<sup>17</sup>.

Clinical data show particularly low levels of zinc in the serum of patients with mood disorders, in whom there is normalisation of serum zinc levels after successful treatment with antidepressants<sup>11, 12</sup>. There are also some preliminary data suggesting that zinc supplementation may enhance antidepressant therapy in patients with unipolar depression<sup>5</sup>. Zinc supplementation significantly reduced the scores in both, Hamilton Depression Rating Scale (HDRS) and Beck Depression Inventory (BDI), measured after 6- and 12-week supplementation when compared with placebo treatment;

these findings are the first demonstration of the benefits of zinc-supplementation in antidepressant therapy<sup>12</sup>.

In general, there is much more data and studies on the effects of zinc on the immune system and peripheral tissues in the literature<sup>18</sup>, while the central and behavioural effects are less well understood<sup>19</sup>. The aim of our study was to examine the behavioural effects of zinc and the effect of single and repeated application of zinc on the behavioural parameters (stillness and locomotor activity of animals, in terms of sniffing, rearing, grooming and locomotion), and antidepressant and anxiolytic effects of zinc in animals. We used zinc histidine dehydrate, as an experimental substance, for which the data in the literature suggest the optimal kinetics in terms of biological activity and bioavailability of the substance<sup>20</sup>.

## Methods

The study included male Wistar albino rats with the body mass of 180–250 g. The animals were kept in clear plastic cages and had ad libitum access to food and water. The room temperature was 22 ± 1°C, with a relative humidity of between 40 to 70% and a 12-hour daily cycle of light and dark, with the lit beginning at 6.00 am. The experiment respected the Ethical Committee codex for work with the experimental animals of the Faculty of Medicine, University of Belgrade. The experiment was performed during the dark period of the daily cycle.

The research included altogether 84 animals, randomly divided into 3 groups of 28, and then within each group another 4 subgroups were made. The effects of zinc were followed using the active ingredients of zinc histidine dehydrate [Zn (His)<sub>2</sub>]. The first subgroups of each group received the solvent (distilled water), and the three others a solution of Zn (His)<sub>2</sub> in increasing doses (10 mg/kg, 20 mg/kg, 30 mg/kg). The substances were administered intraperitoneally (i.p) in the lower right quadrant of the abdomen.

### *Elevated plus maze*

Elevated plus maze (EPM) represents the most widely used animal model for examining anxiety<sup>21</sup>. The maze was elevated to the height of 1 m and consisted of 4 arms (dimensions: 50 × 10 cm). Two opposite arms were closed, and the other two open. There was a central platform (5 × 5 cm) on which the experimental animals were initially placed. The system was monitored by a digital camera, placed above the maze. Recording animal activity and processing data after the

test was conducted by a computer software Any-maze Video Tracking System – Stoelting Co., Wood Dale, IL, USA.

The basis of testing was to induce the conflict situation in experimental animals. The rats, namely, prefer dark and in closed spaces they are the safest. On the other hand, their inquisitive nature forces them to explore, so the open arms of the maze are placed in front of them, which are at the same time potentially dangerous places. It has been shown that the substances with anxiolytic action increase the number of entries into the open arms of the maze, and also prolong the time an animal spends in the open.

Testing was conducted within the first group consisting of 28 animals, 30 min after the application of the substance on each animal in the 4 subgroups. The rats were let into the maze and their spontaneous activity was monitored for 5 min.

#### Forced swim test

The forced swim test (FST) using the method of Porsolt et al.<sup>22</sup>, represents the standard screening test for the evaluation of the antidepressant effects of substances. A FST (hand made) consists of a glass cylinder, 45 cm high, 20 cm in diameter. It is filled with water up to the height of 20 cm, at 21–23°C. Testing lasts for 15 min upon placing the animals into the cylinder. The first 5 min mark the habituation of the animals in the water environment. During the next 10 min the immobility time is measured. That is the time the rats spend floating in the water, so that at least 3 out of their 4 paws keep still. This condition is considered a reaction of despair and depressiveness. The substances with antidepressant potential prolong the time an animal spends in a struggle to find a way out of the cylinder, and reduce the time of immobility in relation to the control group.

The second group of 28 animals was also randomly divided into 4 subgroups of 7 animals each. Within this group the substances (the solvent and zinc histidine dehydrate in the doses of 10, 20, 30 mg/kg) were applied 30 min before placing rats in the cylinder filled with water. The animals were monitored for the next 15 min.

#### General behavioural parameters

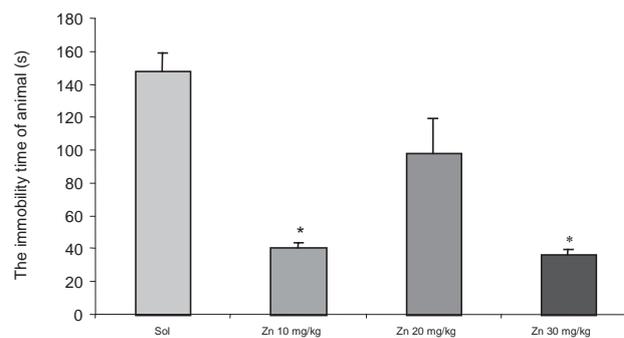
Behavioural parameters were monitored after a repeated application of zinc over 4 days within the third group of 28 animals, randomly divided into 4 subgroups, each receiving a competent substance (the solvent and zinc histidine dehydrate in doses of 10, 20, 30 mg/kg). Two hours after each application the behavioural parameters of each animal were individually measured over 5 min. The important parameters were stillness and the mobility of the animals, in the sense of rearing, sniffing, grooming and locomotion.

#### Statistical data processing

For statistical data processing we used the computer program SPSS 17.0, descriptive statistical method, *t*-test, rank sum test (Mann-Whitney), ANOVA with repeated measurements and the competent software (ANY-maze Video Tracking System – Stoelting Co., Wood Dale, IL, USA). All the numerical data presented in the figures were given as the mean ± SEM.

## Results

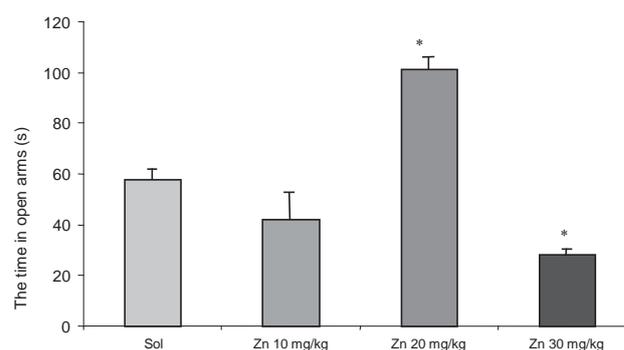
In the forced swim test, the average immobility times of animals, in seconds, for the solvent, Zn (10 mg/kg), Zn (20 mg/kg) and Zn (30 mg/kg) were 147.5, 40.5, 98.0 and 36.5 respectively. It indicates that there is a statistically significant difference between the group with the solvent and the groups with 10 mg/kg and 30 mg/kg of the experimental substances ( $p < 0.05$ ). There was no statistically significant difference for the group with 20 mg/kg of zinc, compared with the control group treated with the solvent (Figure 1).



**Fig. 1 – The immobility time of animals (mean ± SEM) in the forced swim test (FST), after the application of the solvent (Sol) and all the three doses of zinc**

\* $p < 0.05$  vs. Sol – statistically significant difference

In the elevated plus maze, the number of entries into the open arms of the maze was not significantly different between the groups, while there was a statistically significant difference between the groups in the time spent in the open arms of the maze ( $p < 0.05$ ). The animals receiving 20 mg/kg zinc spent significantly more time in the open arms of the maze, indicating an acute anxiolytic effect, while the zinc dose of 30 mg/kg significantly reduced the time the animals spent in the open arms of the maze, indicating a potentially anxiogenic effect (Figure 2).



**Fig. 2 – The time (mean ± SEM) that animals spent in open arms of the elevated plus maze (EPM), after the application of the solvent (Sol) and all the three doses of zinc**

\* $p < 0.05$  vs. Sol – statistically significant difference

Analysis of the data obtained during the investigation of behavioural parameters during the 4-day experiment determined that on the first day there was a statistically significant

difference between the groups that received the solvent and the experimental substance at a dose of 10 mg/kg in terms of increased locomotor activity,  $p < 0.05$ ; the groups that received 20 mg/kg and 30 mg/kg of experimental substances did not show a statistically significant difference as compared with the control group. However, on the second day of the experiment, there was a tendency to reduce spontaneous locomotor activity among those animals that received zinc at the dose of 30 mg/kg ( $p = 0.057$ ). The third and fourth day of testing showed no significant differences in any tested group compared with the control group which received the solvent.

## Discussion

The study animals were tested by FST 30 min after applying the appropriate substance. A statistically significant reduction in immobile time was found among the animals receiving zinc as compared with the group treated with the solvent, thus confirming the antidepressant properties of zinc. Antidepressant effects are especially significant when applying the lowest and highest doses of zinc (10 and 30 mg/kg). These antidepressant effects of zinc are consistent with the results of several previous studies<sup>5, 23–25</sup>. The literature gives different information about the potential mechanisms of action by which Zn exerts antidepressant effects. Antidepressant activity is mainly associated with the inhibition of glutamate NMDA and alpha-amino-3-hydroxy-5-methyl-isoxasoleprapionic acid (AMPA) receptors and an increase in brain-derived neurotrophic factor (BDNF) gene expression in the hippocampus<sup>5, 23, 24</sup>. Some studies suggest an interaction between the serotonergic system and Zn. Zn acts as a selective inhibitor of serotonin reuptake and enhances the pharmacological effects of standard antidepressants<sup>26, 27</sup>.

Besides the zinc influence on the process of glutamate neurotransmission, there are more complex theories about the influence of zinc on GABA-ergic neurotransmission. Specifically, certain subtypes of GABA<sub>A</sub> receptors have specific binding sites for zinc and most studies suggest a possible inhibitory effect of zinc on GABA-ergic neurotransmission<sup>7</sup>. However, the data from molecular studies show that zinc has bidirectional modulatory effects on specific GABA receptors, which are mostly represented in the hippocampus. In this way, zinc is probably included in the process of GABA-ergic neuron plasticity, depending on the neurons' sensitivity to the zinc effect and also depending on the influence of glutamate neurotransmission<sup>28</sup>. Our study showed a dose-dependent bidirectional effect of zinc in experimental model of anxiety (EPM). The animals receiving 20 mg/kg of zinc spent significantly more time in the open arms of the maze, indicating an acute anxiolytic

effect, while the doses of 30 mg/kg zinc significantly reduced the time animals spent in open space, indicating a potential anxiogenic effect.

During the 4-day tracking of the behavioural characteristics of the animals, we followed the parameters of locomotor activity among the rodents (rearing, sniffing, grooming and locomotion). Our results indicate that acute application of zinc on the first day of the test, at the dose of 10 mg/kg, significantly increased locomotor activity of the animals. The zinc dose of 20 mg/kg and 30 mg/kg acutely applied did not significantly affect the locomotor activity of the animals. However, the second day of the experiment showed a reduced spontaneous locomotor activity of animals receiving zinc at the dose 30 mg/kg, while zinc 10 mg/kg and 20 mg/kg had no significant effect on locomotor activity. These dose-dependent bidirectional effects were previously described with Zn effects on memory formation in animals<sup>29</sup>, where lower doses of zinc show some promnesic effects, while higher doses inhibit the formation of memory. On the third and fourth day of testing there were no significant differences in any tested groups compared with the control group. The lack of influence of the third and fourth day can be explained, on the one hand, by the development of some form of tolerance to the substance, while on the other hand it is possible that after repeated applications some adaptive mechanisms start to be active. According to previous studies that negate the formation of tolerance to certain effects of zinc<sup>16</sup>, it is most likely that repeated application of zinc increases zinc excretion through the kidneys, and its effect on locomotor activity is missing.

## Conclusion

All the results of the study suggest that zinc exerts significant effects on the central nervous system. After single application, any doses of zinc showed antidepressant effects. Zinc effects on anxiety and locomotor activity showed dose-dependent bidirectional modulatory effects. The lowest applied dose (10 mg/kg) acutely increased locomotor activity, without effect on anxiety. Zinc 20 mg/kg did not significantly affect locomotor activity, but showed the anxiolytic effects. The largest applied zinc dose (30 mg/kg) showed quite a different effect, by reducing locomotor activity and showing anxiogenic potential. Thus, it can be concluded that zinc, as a fine modulator of glutamate and GABA neurotransmission, regulates specific mental functions, especially anxiety-depressive manifestations.

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