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Change of metabolic characteristics of rats organism and their emotional status and motor activity in the open field test under the exposure of Mo and MoO_3 nanoparticles

Elena Vladimirovna Sheida[1], Elena Anatolevna Rusakova[2], Olga Yurievna Sipailova[3], Elena Anatolevna Sizova[1,2], Svyatoslav Valerievich Lebedev[1,2]

[1]Orenburg State University, Pobedy Pr. 13, 460018, Orenburg, **Russia**
[2]Federal Research Center of Biological Systems and Agro-technologies of the Russian Academy of Sciences, 9th Yanvarya St., 29, 460000, Orenburg, **Russia**
[3]Orenburg state medical University, Sovetskaya St., 6,460000, Orenburg, **Russia**

***Correspondence:** elena-shejjda@mail.ru **Received 20-06-2019, Revised: 30-07-2019, Accepted: 12-08-2019 e-Published: 26-10-2019**

Nanoparticles Mo and MoO_3 (NPs Mo and MoO_3) were tested in vivo. With different physico-chemical characteristics, they had an ambiguous effect on the weight of internal organs and emotional, motor activity and tentatively research behavior of animals. The concentration of 1,0 mg/kg Mo NPs contributed to a maximum change in the weight of lungs and kidneys towards their increase, relative to the control sample. The effect of 25,0 mg/kg Mo NPs led to the greatest increase in weight of lungs. The maximum leaps in weight change at a concentration of 1,2 mg / kg of MoO3 NPs were registered among such organs as heart, kidneys and spleen compared to control analogue. Remarkably, the concentration of 29,0 mg/kg of MoO3 NPs contributed to a uniform increase in the weight of organs such as kidney and spleen when compared with the control sample. By the end of the experiment, the indicators of the emotional sphere of activity in the groups treated with nanoparticles of molybdenum continued to decline, as compared with the control by 22% in groups I and II. The level of emotional activity (grooming, defecation) in the experimental groups throughout the experimental study decreased relative to control. Observing rats that were injected with NPs, signs of intoxication of nervous system, such as depression of motor activity and an increase of brain mass, were identified. The results obtained by us testify to the absence of addiction in animals that received molybdenum NPs and its oxide.

Keywords: rats, nanoparticles (NPs) Mo and MoO_3, behavioral activity, open field test, weight organs

INTRODUCTION

The nano-size and unique properties of molybdenum and its oxide nanoparticles significantly extended their field of application, but at the same time they caused great concern regarding the risks to human health and life and the environment (Asadi et al., 2016; Owen et al., 2005). Used as fillers in cosmetics, dental fillers, in production and processing of glass and ceramics, water filters, electrochemical devices and displays, and also as an antimicrobial agent in polymers, Mo and MoO_3 nanoparticles cause systemic disorders in organs, possessing toxic influence on living systems (Fakhri, 2016; Ema, 2010; Nordberg et al., 2014; Duffin et al., 2007; Jones et al., 2009).

The peculiarities of nanoparticles gradually led to their large-scale use. Molybdenum trioxide nanoparticles penetrating into lymphatic and circulatory systems eventually penetrate into the organs and tissues of body reducing body weight in rats (Fardin, 2016).

Questions about the biological effects of nanoparticles and metals and their oxides remain open up to now. There is no consensus on the mechanisms of damaging effect of nanoscale materials on cells and tissues.

It should be noted that these studies are rather contradictory; therefore, the purpose of our research was to study the overall effect of Mo and MoO3 NPs on growth and development of internal organs of rats, and to study the peculiarities of changes in the orienting behavioral and emotional activities of animals.

MATERIALS AND METHODS

Biomedical studies were performed on 300 white Wistar male rats weighing 110-180 g. Before the experiment, the animals were kept in the experimental biological clinic of the Federal State Budgetary Educational Institution of Higher Professional Education "Orenburg State University", animals were fed with a standard diet for laboratory animals (GOST R 50258-92) according to the rules of laboratory practice for conducting preclinical studies in the Russian Federation (GOST 3 51000.3-96 and 51000.4-96). The experiments were carried out in accordance with the requirements of animal welfare (Griffitt, 2007; Karkishenko et al., 2010).

2.1 Characteristics of the used nanoparticles of Mo and MoO_3

Mo and MoO3 NPs were used as sources of trace elements (Table 1)

Both samples were obtained by plasma chemical synthesis. Particle sizes were estimated based on specific surface measurements, using the Sorbi®-M instrument (Meta®, Russia). The microstructure of powders was analyzed using a Philips CM-30 transmission electron microscope (Philips, Japan). To determine the phase composition, a Rigaku D / MAX-2200VL / PC diffractometer (Rigaku, Japan), Cu Kα radiation was used. Mo NPs in their composition contained at least 99,7% Mo and 0,30% O2; 50,0 ± 0,56 nm in size, with a specific surface area of 14,0 m^2/g. MoO3 NPs contained 99.8% MoO_3 and 0,20% O_2; 92,0 ± 0,54 nm in size, with a specific surface area of 12,0 m^2/ g.

Obtaining lyosols, water suspensions of Mo and MoO_3 were treated with ultrasound using a UZDN-2T disperser (AcademPribor, Russia) at 35 kHz, 300/450 W, 10 µA for 30 minutes. Lyosols of NPs was once administered intraperitoneally.

2.2 Experimental animals and doses

All experimental animals were divided into five groups (n = 6). All animals were kept in the same conditions, fed with the same diet. Control and experimental groups were formed from animals of the same age. Variation in groups according to the initial weight did not exceed 10%. Animals of groups I and II were once intraperitoneally injected with Mo NPs at a dose of 1,0 mg/ kg and 25,0 mg/kg; animals of groups III and IV— with MoO_3 NPs at a dose of 1,2 mg/kg and 29,0 mg/kg. The control group of animals was injected with isotonic sodium chloride solution of 0,9% in equivalent volume. The selected concentrations of nanoparticles did not exceed the maximum tolerated dose (MTD) for this metal. Weighing animals was carried out every three days during the entire study period. At the end of the experiment (14th day), rats were decapitated under Nembutal anesthesia. After this, anatomical cutting and weighing of the internal organs of rats were performed.

2.3 Observation and autopsy

The growth of animals was monitored every morning by individual weighing before feeding (± 2 g). Based on these weightings, the absolute and average daily gains and ratio of studied organs to body weight were calculated. The biomaterial for the study was obtained after decapitation of rats under Nembutal anesthesia (5 animals in each variant of the experiment and in the control on days 1, 7 and 14. Then animals were dissected, anatomical cutting was performed (bones, skin, skeletal muscles, internal organs) with subsequent weighing, grinding and forming an average sample (10 g) for each animal.

Table 1 : Characteristics of Mo and MoO3 NPs used

Number of Sample	NPs	Specific surface area, m^2/g	Size, Nm	Phase composition, %	
				Mo	O_2
1	Mo	14,0	50,0±0,56	99,7	0,30
2	MoO_3	12,0	92,0±0,54	99,8	0,20

2.4 The study of behavioral activity

Emotional, motor activity and orienting exploratory behavior of rats was investigated in the open field test (Jackson et al., 1982; Hall, 1936). The number of different behavioral reactions was counted: Horizontal Motor Activity (HMA) is the number of crossed squares around, by 2/3 and in the center of site; Vertical Motor Activity (VMA) - the number of rearing onto its hind legs; Orienting exploratory activity is the number of peeking into the openings of the field; the emotional factor is the number of fecal boluses; grooming - the number of washes, combings and other elements of care.

2.5 Statistical analysis

The main data obtained in the studies were processed using Excel and Statistica 6.0.

RESULTS AND DISCUSSION

3.1 Effect of Mo and MoO3 NPs on the weight of internal organs

The concentration of 1,0 mg/kg Mo NPs contributed to a maximum change in the weight of lungs and kidneys towards their increase by 14,9 and 7,22%, relative to the control sample. In addition, among the general trend of increasing weight of organs, the weight of spleen and heart increased by 14,6 and 10,5% when compared with the control (Fig 1A).

The effect of 25,0 mg/kg Mo NPs led to the greatest increase in weight of lungs, it was 48,3% more than in the control. A decrease in the weight of spleen, heart, and kidneys was observed after the exposure with such concentration of Mo NPs by 19,1; 17,9 and 8,33% when compared with the control analogue (Fig 1B).

The maximum leaps in weight change at a concentration of 1,2 mg / kg of MoO3 NPs were registered among such organs as heart (by 41,8%), kidneys (29,3%) and spleen (22,8%) compared to control analogue. A slight increase in weight of lung was found by 4,33% relative to the control analog (Fig 1C).

Remarkably, the concentration of 29,0 mg/kg of MoO3 NPs contributed to a uniform increase in the weight of organs such as kidney and spleen by 5,93 and 2,03% when compared with the control sample. However, the maximum weight of lungs increased (by 14.5%), relative to the control. The concentration of MoO_3 NPs in the amount of 29,0 mg/ kg led to decrease in heart weight by 3,51% in contrast to the control analog (Fig 1D).

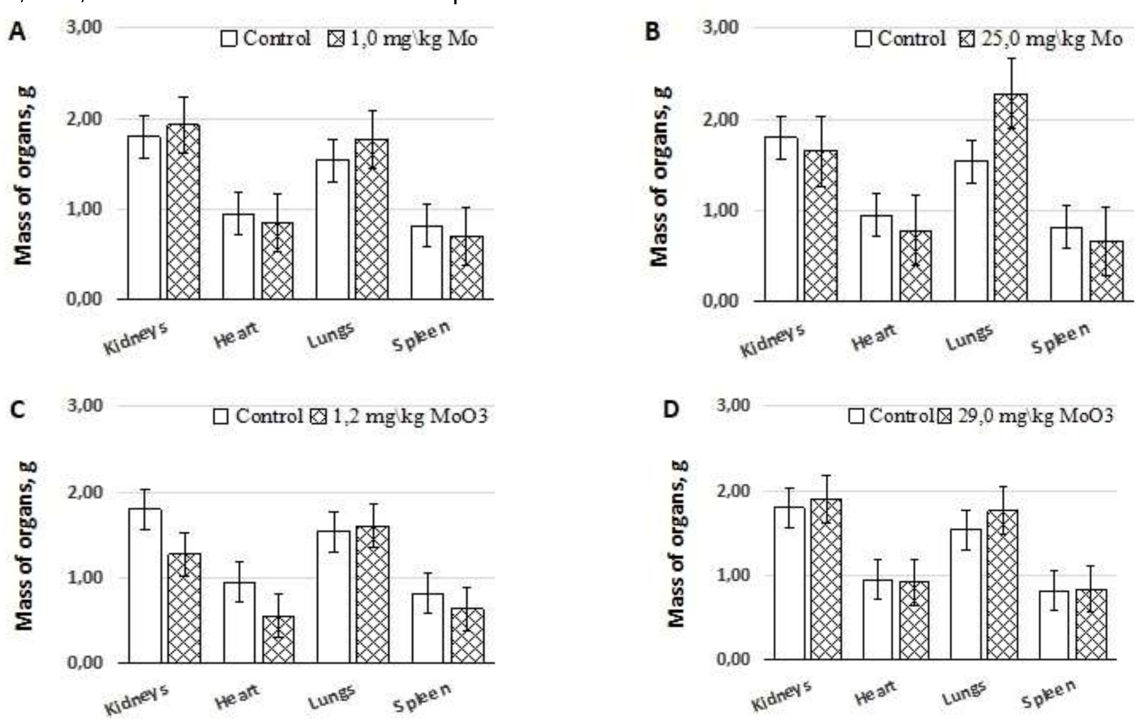

Figure 1: The change in the mass of internal organs of rats after the exposure with different concentrations of Mo and MoO3 NPs: A) 1.0 mg/kg Mo NPs. B) 25.0 mg/ kg Mo NPs. C) 1.2 mg/ kg MoO_3 NPs. D) 29.0 mg/kg MoO_3 NPs.

3.2 Effect of Mo and MoO_3 NPs on Body Weight (BW), Brain Weight (BrW) and the Ratio of Brain Weight to Body Weight (BrW/BW) of Rats

Analyzing BrW, it was established that the concentration of 1,0 mg/kg of Mo NPs (Group I) contributes to the maximum increase of this indicator by 10,9% relative to the control. Mo and MoO_3 NPs at a concentration of 25,0 and 29,0 mg / kg (Group II and IV) led to a steady increase in BrW by 3,85 and 5,49%, it cannot be said about the effect of 1,2 mg/kg of MoO_3 NPs (Group III), it was accompanied by a decrease in the BrW mass by 1,10% when compared with the control analogue (Fig 3B).

Maximum BrW/BW was observed at a concentration of 1,0 mg/kg of Mo NPs (group I), it was by 13,4% higher in quantitative terms compared to the control analogue. The increase of BrW/BW was maintained at a concentration of 25,0 mg/kg Mo NPs (group II) (by 11,7%), 1,2 and 29,0 mg/ kg MoO_3 NPs (group III and IV) (by 5,67 and 7,11%), relative to the control analog (Fig 3B).

3.3 The study of behavioral activity

Testing animals in the “Open field” after the 1st day of Mo nanoparticles introduction, the horizontal motor activity of animals was lower in group II (dose 25 mg/ kg) by 25%, and in group I by 16% in relation to control. However, against the background of a decrease in HMA, VMA almost doubled in groups under the action of nanoparticles. Orienting activity was also reduced in groups; in group I was almost 5 times lower than in the control group. Introducing MoO_3 nanoparticles at a dosage of 1,2 mg/kg to rats in the Open Field test, the motor activity decreased from 1 day to 14 days, and this decrease occurs both in relation to the control and within the group. As a result, the following trend was revealed: on the first day of the experiment, HMA decreased by 30%, but amid a decrease in HMA, VMA increased 2,4 times, and the orienting activity increased almost 2 times relative to the control values (Fig 3A).

On the 7th day of the experiment in group III, HMA and VMA decreased significantly by 23% and 61%, respectively. A similar trend was detected on the 14th day of experiment: HMA decreased significantly by 25%, while the indicators of VMA and orienting activity, on the contrary, increased. It should be noted that motor activity in the control group on day 1 of experiment is higher, but gradually decreases up to the 14^{th} day, it shows the effect of addiction at repeated presentation of experiment.

On day 7 after the introduction of nanoparticles, a decrease of activity indicators was observed in the experimental groups relative to control. The lowest level of motor activity was observed in group II: HMA by 16%, VMA by 64% relative to control. In group I, relative to HMA control, decreased by 7%, VMA by 48%, and aperture examination by 25% (Fig 3 B).

It should be noted that with the introduction of MoO_3 nanoparticles at a dosage of 29 mg / kg, the level of motor activity decreased from 1 day to 14 days, while on the 14th day after the introduction of nanoparticles the lowest activity level was registered for the whole experiment. In animals, the IV group of motor activity was lower for the whole day of the study, both with respect to control and relative to group III.

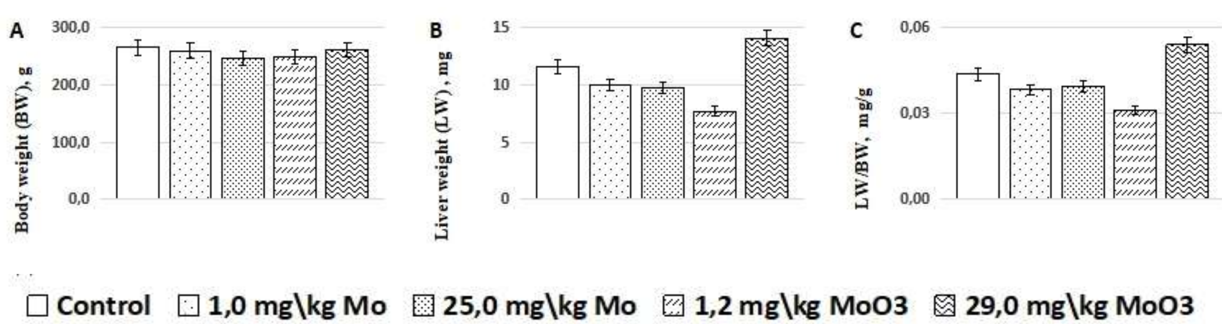

Figure 3 – Quantitative analysis of body weight (A), brain weight (B), ratio of brain weight to body weight (C) of rats

With the introduction of molebdenum nanoparticles into the body 14 days after the introduction, the picture changed significantly. As a result, motor activity in the control group significantly decreased with respect to 1 and 7 days according to all tested indicators in this test, it is possible as a result of adaptation of animals to changing environmental factors. In the experimental groups, motor activity, on the contrary, increased after 1 and 7 days, and also increased relative to the control values, to a greater degree in group II. At the same time, HMA was higher by 41% in group II and 32% in group I, VMA by 50% and 86%, respectively (Fig 3C).

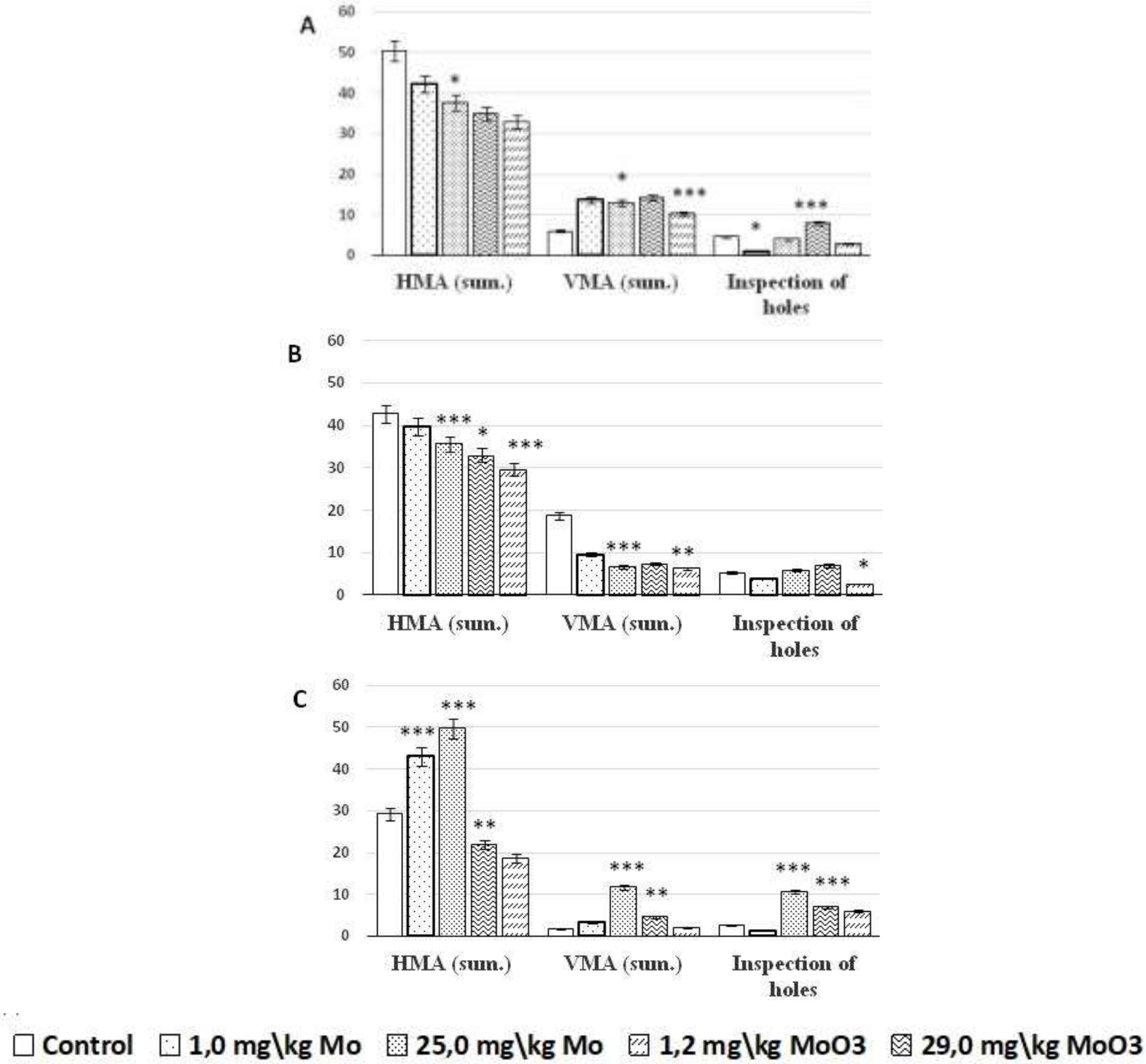

Figure 6 ; Changes in the total motor activity of rats (HMA (sum), VMA (sum) and inspection of holes) after the administration of various concentrations of Mo and MoO3 NPs: A) 1 day of exposure. B) 7 day of exposure. C) 14 day of exposure.

The level of anxiety and fear on day 1 of study was higher in the control group, and the lowest in group I (by 86%). Grooming in group II was 89% lower than in the control group. On the seventh day of the experiment, the picture did not change; the emotional factor was lower by 32% in I and by 48% in group II relative to control. The overall indicators of grooming are lower than in control by 83% in group I and by 92% in group II. By the end of the experiment, the indicators of the emotional sphere of activity in the groups treated with nanoparticles of molybdenum continued to decline, as compared with the control by 22% in groups I and II.

The level of emotional activity (grooming, defecation) in the experimental groups relative to the control on the first day of the experiment decreased by 52% in the III and 69% in the IV groups. On the seventh day of study, there was also a decrease in the emotional activity, and it should be registered that, just a day after introduction of nanoparticles of molybdenum oxide, a decrease was observed in animals that were injected with MoO_3 in a lower dosage. On the 14th day after the introduction of nanoparticles, the emotional factor both in the III and IV groups continued to decline, relative to control, by 33% and 22%, respectively (Fig 8A, 8B, 8C).

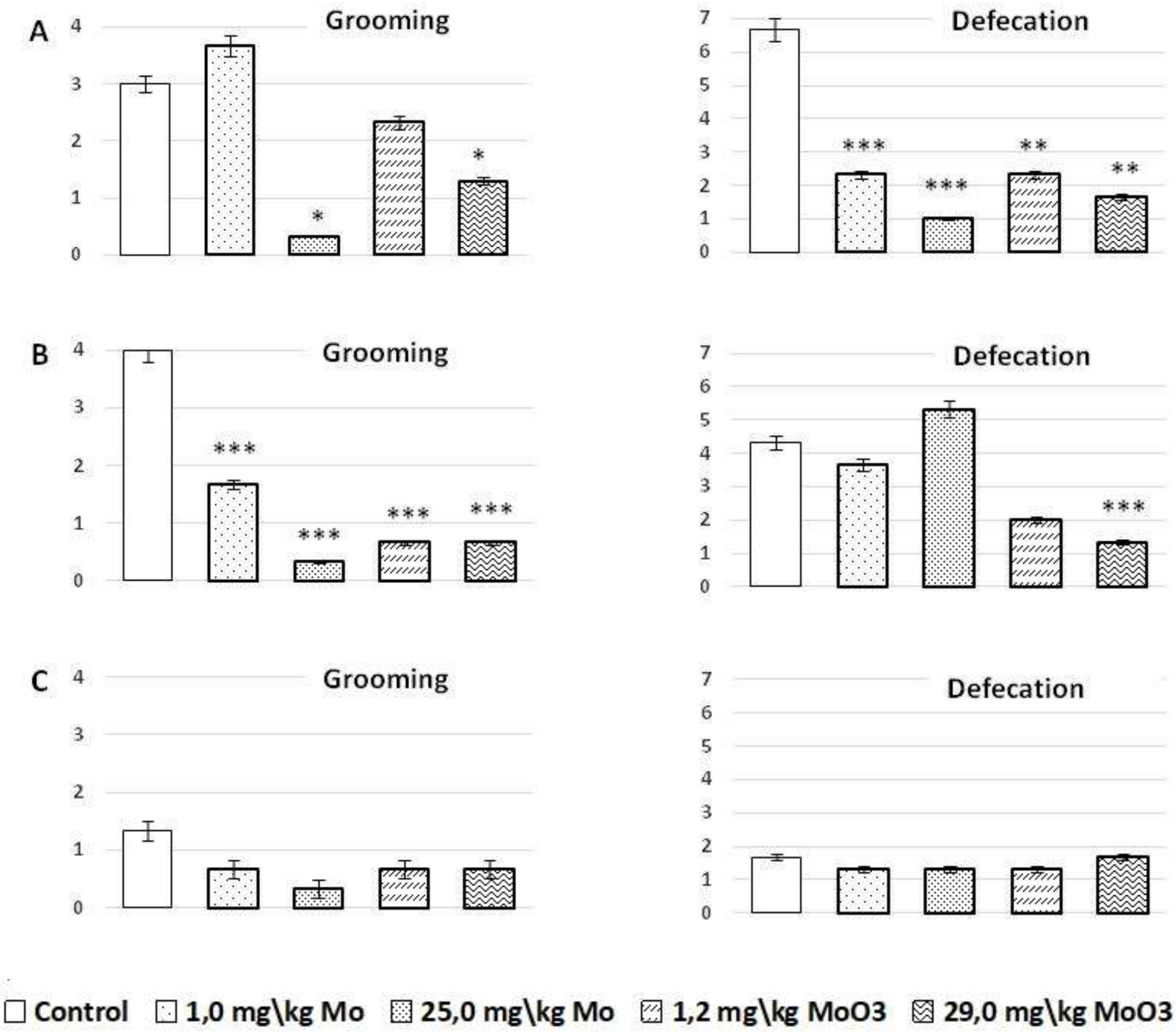

Figure 8; Changes in the emotional state of rats (grooming and defecation) after the administration of various concentrations of Mo and MoO_3 NPs: A) 1 day of exposure. B) 7 day exposure. C) 14 day exposure.

Possessing a high penetrating ability, NPs affect organs and systems of body, nervous system is one of the most susceptible to various system effects. Previously in our works we showed the toxic effects of nanoparticles of iron, titanium and titanium dioxide on the manifestation of cognitive functions in animals and morphological structure of brain. It confirms the data of other researchers on the increase of absolute weight of brain and the change in the emotional state of animals under the influence of titanium dioxide NPs (Amara et al., 2013; Sheida et al., 2017; Krivova et al., 2011).

Observing rats that were injected with NPs, signs of intoxication of nervous system, such as depression of motor activity and an increase of brain mass, were identified. The results obtained by us testify to the absence of addiction in animals that received molybdenum NPs and its oxide.

CONCLUSION

Thus, in groups receiving NPs of molybdenum, the level of general motor activity and the emotional factor decreased on day 7 of the experiment, and by 14 day, on the contrary, increased, indicating the predominance of excitation processes over inhibition processes. It should be noted that the behavior of the control animals in the process of testing became calmer, which indicates a decrease in the stress level of the animals, as evidenced by a decrease in all indicators of motor and emotional spheres of activity. In test groups, against the background of motor activity increase, the level of emotional tension and fear also increases; it is probably related to the accumulation of NPs in the brain and the formation of a new level of functional activity.

With the introduction of MoO_3 NPs to animals, inhibition of motor activity and emotionality was noted, moreover, in those rats that were injected with LF at a higher dosage and greater exposure time (14 days), which probably indicates the development of a depressive state in rats as a result of LF penetration into the brain.

Based on the results obtained, it can be concluded that Mo and MoO_3 NPs have a toxic effect on the normal functioning of certain body systems. Possessing the neurotoxic effect NPs of molybdenum and its oxide, they alter behavioral reactions and motor activity, and the severity of this effect directly depends on the exposure time and particle dosage.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

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AUTHOR CONTRIBUTIONS

SEV and REA conducted experimental studies on animals, and also wrote a manuscript. SOY selection of tissues and organs for study, analysis of the results. SEA and LSV designed the experiments and reviewed the manuscript. All authors have read and approved the final version.

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