Pakistan J. Zool., vol. 54(6), pp 2857-2864, 2022 DOI: https://dx.doi.org/10.17582/journal.pjz/20210825060829

Ni Oxide Nanoparticles has the Potential to Drastically Affect the Behavior of Albino Mice in a Sex Specific Manner

Malik Fiaz Hussain[1], Atif Akbar[2], Muhammad Asif[1], Laraib Nisar[3], Muhammad Naeem Ashiq[3] and Furhan Iqbal[1]*

[1]*Institute of Pure and Applied Biology, Zoology Division. Bahauddin Zakariya University, Multan, Pakistan*
[2]*Department of Statistics, Bahauddin Zakariya University, Multan, Pakistan*
[3]*Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan.*

Malik Fiaz Hussain and Atif Akbar have made equal contributions in this manuscript.

Article Information
Received 25 August 2021
Revised 02 October 2021
Accepted 15 October 2021
Available online 04 February 2022 (early access)
Published 29 August 2022

Authors' Contribution
FI designed and supervised the project and revised the final manuscript. MNA and LN synthesized and characterized the Ni oxide nanoparticles. MFH, AA and MA maintained the mice colony and performed all mentioned experiments. All data were generated in-house and that no paper mill was used.

Key words
Ni oxide nanoparticles, Rota rod test, Light dark test, Open field test, Novel object recognition test

ABSTRACT

Ni oxide nanoparticles (NiO NPs) are extensively used in industries dealing with welding, electroplating and in production of alloys but despite of their large scale uses, it is expected that they might have lot more drawbacks than their bulky counter parts. Present investigation was aimed to document the effect of two different doses of NiO NPs on behavior of albino mice in a sex specific manner. Five weeks old albino mice (N = 48) of both sex were intraperitoneally injected with either low dose (20 mg) or high dose (50mg/ ml saline/ Kg body weight) of NiO NPs (average particle size 43 nm) for 14 days. Control groups were treated with saline solution. A series of behavioral tests were conducted in all subjects. Both low and high dose of NiO NPs treated male mice, during rota rod test, spent significantly less time on rotating rod than controls. Male mice treated with both doses of NiO NPs performed more stretch attend reflex than control. Female mice injected with high dose of NiO NPs had significantly reduced mobile and immobile episodes during open field test than mice exposed to low dose and control group. High dose of NiO NPs treated females had reduced line crossing, stretch attend reflex, number of approaches to familiar and novel objects than low dose NiO NPs and saline treated female mice. No change in body weight was observed when this parameter was compared between NiO NPs and saline injected albino mice of both sexes. In conclusion, we are reporting that NiO NPs can affect the muscular activity, object recognition capacity and exploratory behaviour of albino mice. The effects were more pronounced at higher dose and in female mice.

INTRODUCTION

Ni (Ni) is considered as non essential metal for human and mammals but as we are exposed to variety of Ni containing materials, adulterants and pollutants consequently Ni levels are continuously rising in food and water that we consume. For example, Ni steel alloy is common part of our cookware and they may release Ni into food. Fuel's (containing Ni) consumption is a major source to deposit Ni in our atmosphere (Haber *et al*., 2017). Ni can be directly inhaled along with tobacco smoke and it can get enter in body when our skin comes in contact with shampoos, jewelry, detergents and coins containing Ni in their composition (Cao *et al*., 2019). Ni is known to interact with cellular membranes as well as it can bind to proteins and DNA in a cell affecting their central dogma and gene expression profile. It can be deposited to all levels of organization in an organism and known to be toxic and carcinogenic (Song *et al*., 2017).

Nanoparticles have enabled many analyses and therapies that were not possible before (Murthy, 2007). This is due to the fact that nanoparticles which have changed electrical conductivity, hardness, chemical reactivity and biological activity have higher biochemical activity (Ren *et al*., 2009). Same is true for nano NiO that are used in bulks as photo-catalyst, electrocatalyst, in energy devices and in gas sensors due to their semiconductor nature (Zaitseva *et al*., 2016; Cao *et al*., 2019). A couple of in vitro studies have already reported that NiO NPs are e

* Corresponding author: furhan.iqbal@bzu.edu.pk
0030-9923/2022/0006-2857 $ 9.00/0

toxic for mammalian cells (Siddiqui *et al*., 2012; Ahamed *et al*., 2013) but in vivo studies were lacking for NiO NPs. We hypothesized that people working in industries where NiO NPs are used as raw material and the end users of these products are seriously exposed to hazards of these materials. Hence, the current investigation was aimed to report the effect of variable doses of NiO NPs (20 and 50 mg/ Kg body weight) on neuromuscular function, exploratory behavior and on object recognition capability of albino mice in a sex specific manner.

MATERIALS AND METHODS

Experimental animals and design

Albino mice (five weeks old; C57BL/6 strain) were used during this study. Animal housing and colony maintenance conditions were same as reported in Hussain *et al*. (2020). NiO NPs were also generated by hydro thermal method as described previously by Hussain *et al*. (2020).

Mice were kept in rodent cages individually during the experiments and on the basis of specific treatment, they were divided into three groups, each of 16, consisted of equal number of male and female. Group I was injected intraperitoneally with 20mg/ ml saline/ Kg body weight of NiO NPs for 14 days. Group 2 was intraperitoneally injected 50mg/ ml saline/ Kg body weight of NiO NPs for 14 days. Group 3 was intraperitoneally injected with saline solution for 14 days and acted as control. Injection of specific dose was done once per day for all treatments. On daily basis, body weight of each animal was recorded before intraperitoneal injections.

Behavioral testing

On 11th till 14th day of experiments, a battery of behavioral tests were performed in all experimental treatments: rota rod test on day 11, open field test on day 12, light dark box test on day 13 and novel object recognition test on day 14 were performed.

Rota rod test

A locally manufactured rota rod test apparatus was used to compare balance and neuromuscular coordination between three experimental treatments. Apparatus contained a rotating drum that accelerates uniformly with 40 rpm. Each animal first received three training trials after that they faced three experimental trials. Maximum duration to test a mouse was 2 min. Average time spent by each mouse on rotating drum was recorded following Allahyar *et al*. (2016).

Open field test

Open field test was used to report the exploratory behavior of mice under variable dose exposure. Maximum duration of this test was 10 min in which each mouse was observed in an area 40 cm wide x 40 cm long with 70 cm high walls. Parameters like time in corners, time in centre, mobile episodes, immobile episodes, rotations, anti-clockwise rotations and clockwise rotations and stretch attend reflex were recorded for each mouse following Iqbal *et al*. (2017).

Light and dark box test

Light and dark box test is used to study exploratory behavior in rodents. The structure of light/dark box is same as we have previously described in Akram *et al*. (2020). For each mouse, time spent in each (light or dark) chamber, rearing, stretch attend reflex, defecation, urination and the number of transitions between the light and dark chambers were recorded. The maximum test duration was 5 min.

Novel object recognition test

Object recognition capability of albino mice was tested following exposure to NiO NPs. Apparatus consists of a rectangular chamber (40 cm length x 30 cm height x 35 cm width) with two sample objects (A and B). Both objects were placed in the opposite corners of the arena**.** A line was drawn in the center of the floor of apparatus (at 20 cm length) to divide it into two halves. During first trial, each mouse placed in center was allowed to explore two sample objects (A and B) for 5 min. In trial two, the location of objects was unchanged but objects A was replaced by a novel object. Each mouse explored the arena for 5 min. Parameters like line cross, stretch attend reflex, number of approaches to object A, B or novel object, time with A, B or novel object, rearing, urination and defecation were recorded for each animal following Khosa *et al*. (2020).

Statistical analysis

Data is presented as Mean±SEM. Data was analyzed by using Minitab (version 17, Pennsylvania). $P \leq 0.05$ was set as significance level. One-way analysis of variance (ANOVA) was applied to compare the performance of conducted behavioral test between the three experimental treatments followed by Tukey's post-hoc test.

RESULTS

Body weight

One-way analysis of variance test results revealed that during present study, exposure to variable doses of NiO NPs did not affect the body weight on all studied time point in both sex (Fig. 1).

Rota rod test

One-way ANOVA results revealed that male mice injected with both doses of NiO NPs stayed on the

rotating rod for significantly less time than saline injected male albino mice (P= 0.03) (Fig. 2A). One-way ANOVA indicated that rota rod test performance did not vary significantly (P= 0.6) when compared between female mice treated with low and high dose of NiO NPs and saline water treated control group (Fig. 2B).

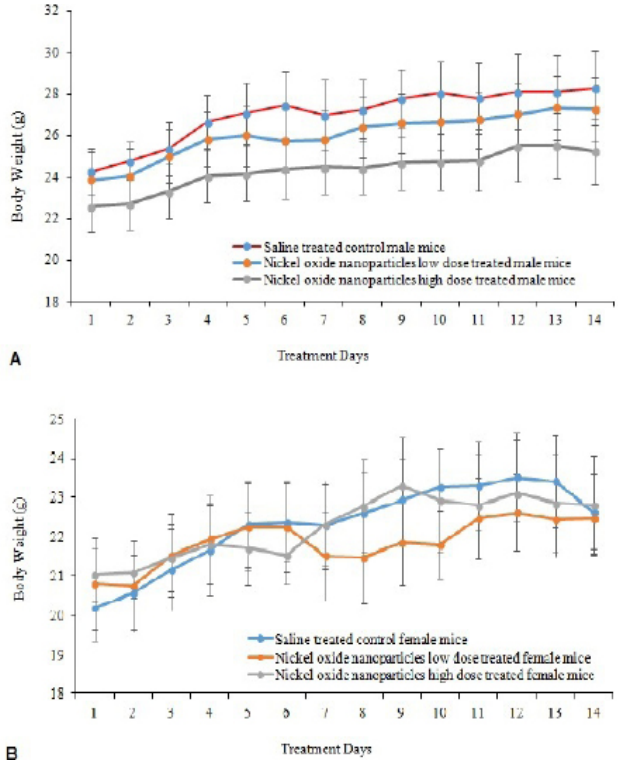

Fig. 1. Comparison of change in body weight between (A) male and (B) female mice intraperitoneally injected for 14 days either with 20 mg or 50 mg/ml saline/ Kg body weight of NiO NPs or with saline solution. Data is expressed as mean ± standard error of mean. P values represent the outcome of two samples t-test calculated between a specific NiO NPs treatment and saline group for each treatment day.

Open field test

One-way ANOVA results revealed that studied open field test parameters remained unaffected (P > 0.05) when compared between the three experimental treatments of male mice (Table I). One-way ANOVA results revealed that frequency of mobile (P= 0.006) and immobile episodes (P= 0.007) was significantly different when compared between low, high dose NiO NPs and saline treated female mice. Analysis of Tukey's post hoc test indicated that female mice exposed to high dose of NiO NPs had significantly reduced mobile and immobile episodes than control group and female mice injected with low dose of NiO NPs (Table I).

Light and dark box test

One-way ANOVA results demonstrated that the only parameter that varied significantly during light dark box between NiO NPs treated and untreated male mice was stretch attend reflex (P = 0.01). Post hoc Tukey's test result indicated that saline treated control had significantly reduced stretch attend reflex than both NiO NPs treated male mice (Table I). While one-way ANOVA results indicated that none of the studied parameters during light and dark box tests reached the statistical significance (P > 0.05) when compared between NiO NPs and saline treated female mice (Table I).

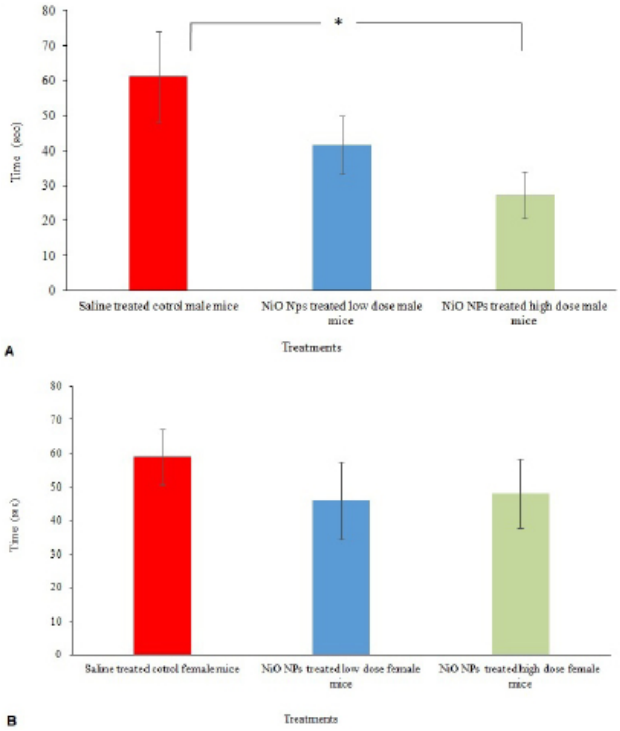

Fig. 2. Effect of 20 mg or 50 mg/ml saline/ Kg body weight of NiO NPs on rota rod test performance between (A) male and (B) female mice injected intraperitoneally for 14 days. Data is expressed as mean ± standard error of mean. P values represent the outcome of two samples t-test calculated between a specific NiO NPs treatment and saline group for studied parameter.

Novel object recognition test

One way analysis of variance analysis indicated that time spends with object A during trial I was the only parameter that was significantly different when compared between NiO NPs and saline treated male mice during novel object recognition test. Post hoc Tukey's test results revealed that male mice injected with 20 mg of NiO NPs remained with object A for more time than high dose

Table I. Effect of low (20 mg/ ml of solvent / Kg of body weight) and high dose (50 mg/ ml of solvent / Kg of body weight) of NiO NPs on various studied parameters of open field test, light dark box test of albino mice. All values are expressed as mean ± standard error of mean. P values represent the results of one way ANOVA followed by post hoc Tukey's test calculated for each studied parameter.

Parameters	Male mice				Female mice			
	Saline treated ♂ mice (n=8)	NiO NPs treatments (♂)		P value	Saline treated ♀ mice (n=8)	NiO NPs treatments (♀)		P value
		Low dose (n=8)	High dose (n=8)			Low dose (n=8)	High dose (n=8)	
Open field test								
Time in corner (sec)	555.3 ± 14 $_A$	544.5 ± 12 $_A$	555.8 ± 12 $_A$	0.8	538.1 ± 11 $_A$	524.4 ± 19 $_A$	569.4 ± 8.3 $_A$	0.08
Center (sec)	44.8 ± 14 $_A$	55.5 ± 12 $_A$	44.3 ± 12 $_A$	0.8	62.1 ± 11 $_A$	75.6 ± 19 $_A$	30.6 ± 8.3 $_A$	0.08
Mobile episode	138.3 ± 16 $_A$	139.8 ± 17 $_A$	128.4 ± 7.7 $_A$	0.8	159.8 ± 6.5 $_A$	148.9 ± 10 $_A$	110.8 ± 13 $_B$	0.006**
Immobile episode	137.8 ± 16 $_A$	139.1 ± 19 $_A$	127.5 ± 7.5 $_A$	0.8	159.5±6.4 $_A$	149.4 ± 10 $_A$	111.5 ± 13 $_B$	0.007**
Clockwise rotation	21.1 ± 2.5 $_A$	24.5 ± 2.5 $_A$	20.1 ± 4.6 $_A$	0.6	29.7±2.4	17.6 ± 3.3	20.9 ± 4.8	0.07
Anticlockwise rotation	18.5 ± 3.2 $_A$	21.5 ± 1.9 $_A$	21.3 ± 4.6 $_A$	0.8	30.9 ± 3.2	22.5 ± 5.0	23.1 ± 4.8	0.3
Light dark box test								
Line cross	5.7 ± 1.1 $_A$	7.1 ± 1.2 $_A$	8.6 ± 1.1 $_A$	0.4	18.6 ± 2.1 $_A$	17.4 ± 1.4 $_A$	14.5 ± 3.1 $_A$	0.4
Transition frequency	14.1 ± 1.8 $_A$	16.2 ± 1.3 $_A$	16.5 ± 1.1 $_A$	0.2	9.1 ± 1.2 $_A$	7.2 ± 0.9 $_A$	5.5 ± 1.3 $_A$	0.1
Rearing frequency	11.7 ± 2.3 $_A$	10.4 ± 1.6 $_A$	17.4 ± 3.7 $_A$	0.2	12.5 ± 2.1 $_A$	9.1 ± 1.0 $_A$	9.1 ± 1.7 $_A$	0.2
Stretch attend frequency	30.9 ± 3.9 $_A$	47.9 ± 5.1 $_B$	46.9 ± 4.1 $_B$	0.01**	45.9 ± 4.0 $_A$	46.8 ± 4.2 $_A$	51.1 ± 9.1 $_A$	0.8
Time in light (sec)	142.5 ± 24 $_A$	148.9 ± 22 $_A$	150.9 ± 14 $_A$	0.9	168.3 ± 17 $_A$	172.5 ± 20 $_A$	161.6 ± 26 $_A$	0.9
Dark (sec)	156. 3 ± 23 $_A$	151.1 ± 22 $_A$	149.1 ± 14 $_A$	0.9	131.8 ± 17 $_A$	127.5 ± 20 $_A$	138.4 ± 26 $_A$	0.9
Defecation	3.1 ± 0.9 $_A$	2.7 ± 0.8 $_A$	1.1 ± 0.6 $_A$	0.1	1.6 ± 0.4 $_A$	1.5 ± 0.7 $_A$	2.1 ± 0.6 $_A$	0.6

P > 0.05 = Non-significant; P ≤ 0.01 = Significant (**)

NiO NPs and saline treated male controls. All other studied parameters during novel object recognition test, trial I and II, remained unaffected (P > 0.05) for male mice (Table II).

One way ANOVA results indicated that for male mice, performance of novel object test trial one remained unaffected (P > 0.05) when compared between NiO NPs treated and untreated female mice (Table II). One way ANOVA results for novel object trial II revealed that line cross (P = 0.03), stretch attend reflex (P = 0.005), number of approaches to novel object (P = 0.01) and number of approaches to object B (P= 0.04) were significantly different significantly when these parameters were compared between all three female experimental treatments. Tukey's post hoc test analysis indicated that female mice injected with 50 mg/Kg body weight of NiO NPs had reduced line crossing, stretch attend reflex, number of approaches with familiar and novel objects as compared to low dose NiO NPs and saline treated female mice (Table II). One way ANOVA analysis revealed that stretch attend reflex (P= 0.05) during trial 1, line cross (P= 0.03), stretch attend reflex (P= 0.005), number of approach to novel object (P= 0.01), number of approach to familiar object (B) (P= 0.04) during novel object recognition test trial two were the parameters that were found significantly different upon comparison between three experimental treatments of female mice (Table II). Tukey's test results revealed that female mice treated with 50 mg/ml saline/Kg body weight of NiO NPs had significantly decreased stretch attend reflex (P= 0.005), line crossing (P= 0.03), number of approaches to novel object (A) (P= 0.01) and number of approaches towards familiar object (B) (P = 0.03) during second trial of novel object recognition test (Table II).

DISCUSSION

NPs are known to cross physiological barriers and to enter in blood vascular system from where they can be transported to almost all organs and tissues disturbing their normal functioning (Oberdörster *et al*., 2005). For Ni toxicity, two factors are critical; first how it is applied to an organism and second how much the applied Ni compound is soluble in physiological solutions (Das *et al*., 2008; Liapi *et al*., 2011). For NPs, it is an established fact that larger particles having diameter 200 nm or higher can easily be neutralized by the spleen and ultimately removed by the phagocytotic cells decreasing the concentration

Table II. Effect of low (20 mg/ ml of solvent/ Kg of body weight) and high dose (50 mg/ ml of solvent / Kg of body weight) NiO NPs on various studied parameters of novel object recognition test (trial 1 and II) of male and female albino mice. All values are expressed as mean ± SEM. P values represent the results of one-way ANOVA followed by post hoc Tukey's test calculated for each studied parameter.

Parameters	Trial I				Trial II			
	Saline treated mice (n=8)	NiO NPs treatments		P value	Saline treated mice (n=8)	NiO NPs treatments		P value
		Low dose (n=8)	High dose (n=8)			Low dose (n=8)	High dose (n=8)	
Male mice								
Line cross	24.1 ± 1.6 $_{A}$	19.1 ± 1.3 $_{A}$	20.9 ± 1.4 $_{A}$	0.06	18.7 ± 0.8 $_{A}$	26.1 ± 4.9 $_{A}$	17.4 ± 1.8 $_{A}$	0.1
Stretch attend reflex	40.4 ± 5.3 $_{A}$	39.25 ± 2.1 $_{A}$	32.1 ± 3.7 $_{A}$	0.3	36.8 ± 3.7 $_{A}$	26.9 ± 4.1 $_{A}$	27.1 ± 4.7 $_{A}$	0.1
No. of approach to object A	8.2 ± 1.8 $_{A}$	11.7 ± 1.1 $_{A}$	9.7 ± 1.3 $_{A}$	0.3	6.63 ± 0.8 $_{A}$	8.5 ± 0.8 $_{A}$	6.2 ± 0.8 $_{A}$	0.1
Object B	7.6 ± 1.1 $_{A}$	9.1 ± 3.5 $_{A}$	6.7 ± 0.8 $_{A}$	0.3	6.1 ± 1.1 $_{A}$	7.2 ± 1.3 $_{A}$	4.9 ± 0.6 $_{A}$	0.3
Time spent with object A (s)	**54.9 ± 1.1 $_{A}$**	**86.1 ± 8.3 $_{B}$**	**81.1 ± 5.2 $_{A}$**	**0.03***	41.3 ± 6.6 $_{A}$	61.1 ± 9.6 $_{A}$	43.4 ± 9.9 $_{A}$	0.2
Object B (s)	79.1 ± 16	57.6 ± 8.5	75.1 ± 20	0.4	52.4 ± 7.5 $_{A}$	45.8 ± 8.9 $_{A}$	53.9 ± 10 $_{A}$	0.8
Rearing frequency	11.7 ± 3.1	8.9 ± 1.2	6.8 ± 0.8	0.3	8.8 ± 2.3 $_{A}$	7.1 ± 1.5 $_{A}$	10.8 ± 0.9 $_{A}$	0.2
Defecation	1.5 ± 0.5	1.7 ± 0.7	0.6 ± 0.5	0.4	1.8 ± 0.6 $_{A}$	1.1 ± 0.5 $_{A}$	1.4 ± 0.7 $_{A}$	0.6
Female mice								
Line cross	29.3 ± 4.4 $_{A}$	22.3 ± 1.8 $_{A}$	20.1 ± 2.1 $_{A}$	0.1	**27.8 ± 4.1 $_{A}$**	**26.2 ± 1.7 $_{A}$**	**16.7 ± 2.3 $_{B}$**	**0.03***
Stretch attend Reflex	54.5 ± 6.4 $_{A}$	46.1 ± 4.9 $_{A}$	34.4 ± 5.8 $_{A}$	0.06	**46.8 ± 5.7 $_{A}$**	**38.5 ± 4.4 $_{A}$**	**22.5 ± 4.2 $_{B}$**	**0.005****
No. of approach to object A	11.6 ± 1.6 $_{A}$	12.2 ± 1.2 $_{A}$	8.5 ± 1.9 $_{A}$	0.2	**10.8 ± 1.2 $_{A}$**	**9.75 ± 0.8 $_{A}$**	**6.38 ± 0.9 $_{B}$**	**0.01***
Object B	6.2 ± 1.2 $_{A}$	9.5 ± 1.3 $_{A}$	8.5 ± 1.5 $_{A}$	0.2	**8.6 ± 1.5 $_{A}$**	**7.3 ± 0.9 $_{A}$**	**4.5 ± 0.7 $_{B}$**	**0.04***
Time spent with object A (s)	103.9 ± 17 $_{A}$	81.4 ± 7.8 $_{A}$	64.8 ± 14 $_{A}$	0.1	61.5 ± 6.2 $_{A}$	73.8 ± 17 $_{A}$	72.9 ± 23 $_{A}$	0.8
Object B (s)	55.5 ± 7.1 $_{A}$	64.4 ± 8.6 $_{A}$	87.6 ± 20 $_{A}$	0.2	88.8 ± 15 $_{A}$	61.0 ± 9.5 $_{A}$	51.8 ± 13 $_{A}$	0.1
Rearing frequency	10.2 ± 2.7 $_{A}$	10.1 ± 1.6 $_{A}$	13.9 ± 3.6 $_{A}$	0.5	8.00 ± 1.6 $_{A}$	7.0 ± 1.0 $_{A}$	7.25 ± 1.4 $_{A}$	0.8
Defecation	1.5 ± 0.4 $_{A}$	0.7 ± 0.3 $_{A}$	1.4 ± 0.6 $_{A}$	0.4	2.4 ± 0.3 $_{A}$	2.6 ± 0.6 $_{A}$	2.0 ± 0.4 $_{A}$	0.6

P > 0.05 = Non significant; P ≤ 0.05 = least significant (*); P ≤ 0.01 = Significant (**). Object A was replaced by novel objet in trial II.

of these chemicals in blood and hence their distribution to other organs. Smaller NPs having diameters 10 nm or less have rapid renal clearance. NPs in size range of 10 to 100 nm are most commonly used for intravenous injection and they are known to remain in blood circulation for extended duration and can reach to tissue/cellular levels of an organism (Akhtar *et al*., 2018). The NiO NPs that were used in the present studies had an average diameter of 43 nm, calculated using Scherer formula (Hussain *et al*., 2020). This diameter of NiO NPs indicates that such small size NPs have more chances to be transported to brain and to affect the behavior.

Although, harmful effects of Ni are well established but at the same time they are in extensive industrial use as well, so it would be worth determining that what are the levels beyond which this metal gets toxic for living systems (Sadiq *et al*., 2012). There are a number of studies in which various forms of Ni NPs has been applied to experimental animals with dose concentrations varying between 2 to 500 mg/Kg body weight but the effect of 20mg and 50 mg/Kg NiO NPs were never investigated in mice before. So we used these doses to investigate their effects on selected aspects of albino mice behavior.

It has been an established fact that sex of an animal influences its cognitive functions like perception, emotion and memory formation (Cahill, 2006). This is due to the fact that brain structure and functions have sex specific reported differences (Xin *et al*., 2019). The gender-related morphological differences are already reported for a number of brain regions including the corpus callosum (Prendergast *et al*., 2015), cerebellum (Raz *et al*., 2001) and limbic-thalamo-cortical circuitry (Xin *et al*., 2019). This has led us to hypothesize that same dose of applied NiO NPs may induce sex specific effects in albino mice.

It was observed during present study, that exposure to high dose of NiO NPs had significantly disturbed the exploratory behavior, neuromuscular coordination and object recognition capacity of albino mice. These findings

are in line with Ijomone *et al*. (2018) who had intraperitoneal injected rats for 21 days with 5, 10 and 20 mg/Kg $NiCl_2$ and reported that memory formation during Y-maze and exploratory behavior open field test, respectively, were compromised. They had documented that administration of Ni had affected neuronal morphology and neurons of hippocampus and striatum were seriously damaged and both these brain areas are associated with cognition and motor activity. They had suggested that increased oxidative stress due to Ni exposure caused these behavioral impairments. NPs are known to cause oxidative stress upon application to living systems (Akhtar *et al*., 2018) and same is true when we discuss the neurotoxicity caused by Ni NPs as they have potential to change the neuronal membrane compositions and disturbing their functions all together (Lamtai *et al*., 2018). Similar to our observations, He *et al*. (2013) had documented that exposure to Ni disturbed spatial memory (associated with site recognition) and exploratory behavior of mice. He *et al*. (2013) found that applied Ni got deposited in the cerebral cortex of mice, part of brain that has critical role in memory formation, disturbing its morphology and physiology. Our novel object recognition test results are in line with those of Rabelo *et al*. (2016) who had applied tannery effluent to Swiss mice for 15 days. The effluent was rich in heavy metals like Ni, chromium and cadmium. It was observed that the capacity of mice to recognize novel object was significantly reduced when they were exposed to the effluent. Nation *et al*. (1985) had also reported that rats treated with 20 mg/kg $NiCl_2$ for 14 days pressed lever to get food at a significantly lower rate than controls. Earlier Liapi *et al*. (2011) had reported that short-term $NiCl_2$ administration (13 mg/kg) to adult rat resulted in disturbed release and uptake of neurotransmitters like serotonin, catecholamine and glutamate by the neurons that disturbs the normal brain functioning. Liapi *et al*. (2011) has also been documented that Ni interacts with ion channels and synaptic vesicles in a neuron and affects neurotransmitter release. Many metals (including Ni) are also known to influences cellular signaling pathways that affects metabolism of neurotransmitters (Sadiq *et al*., 2012). It is also reported that divalent cations (including Ni) compete with the agonist of AMPA receptors for the binding sites disturbing their normal functioning as these receptors play important role in synaptic transmission and neural plasticity (Dorofeeva *et al*., 2005). All these observations are justifying the poor neurological test performance by albino mice during present study upon exposure to NiO NPs.

CONCLUSION

In conclusion, we are reporting the drastic behavioural effects in mice following intraperitoneal injections of NiO NPs for 14 days. These NPs can affect the muscular activity, object recognition capacity and exploratory behaviour of albino mice. The effects were more pronounced at higher dose and in female mice.

Ethical approval

Experimental procedures were reviewed and approved by the ethical committee of Bahauddin Zakariya University Multan (Pakistan) at Institute of Pure and Applied Biology via application number Biol/Ethics/2018-39.

Consent to participate

Not applicable.

Consent to publish

Authors are giving their consent to publisher that they can publish this data upon acceptance of our manuscript.

Funding

No specific funding was involved in this project.

Availability of data and materials

All the data generated during this project will be made available upon request.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ahamed, M., Ali, D., Alhadlaq, H.A., and Akhtar, M.J., 2013. Ni oxide nanoparticles exert cytotoxicity via oxidative stress and induce apoptotic response in human liver cells (HepG2). *Chemosphere*, **93**: 2514-2522. https://doi.org/10.1016/j.chemosphere.2013.09.047

Akhtar, S., Saba, S., Rehman, S., Hassan, A., Samad, N., Amir, M., Akram, M., Ijaz, M., Asif, K., Ashiq, M.N., and Iqbal, F., 2018. Microemulsion-based synthesis of strontium hexaferrite cobalt iron oxide nanoparticles and their biocompatibility in albino mice. *J. exp. Nanosci*., **13**: 199-210. https://doi.org/10.1080/17458080.2018.1475759

Akram, I.N., Akhtar, S., Khadija, G., Latif, M., Noreen, A., Mobeen, M., Sajjad, F., Sardar, Z., Iqbal, S., Ashiq, M.N., and Iqbal, F., 2020. Synthesis, characterization and biocompatibility of Lanthanum Titanate nanoparticles in albino mice in a sex specific manner. *Naunyn-Schmiedeberg's Arch Pharmacol*., **393**: 1089-1101. https://doi.org/10.1007/s00210-020-01819-z

Allahyar, R., Akbar, A., and Iqbal, F., 2016. Effect of creatine monohydrate supplementation on learning, memory and neuromuscular coordination in female albino mice. *Acta Neuropsy*., **29**: 27-34. https://doi.org/10.1017/neu.2016.28

Cahill, L., 2006. Why sex matters for neuroscience. *Nat. Rev. Neurosci*., **7**: 477–484. https://doi.org/10.1038/nrn1909

Cao, S., Peng, L., Han, T., Liu, B., Zhu, D., Zhao, C., Xu, J., Tang, Y., Wang, J., and He, S., 2019. Hydrothermal synthesis of nanoparticles-assembled NiO microspheres and their sensing properties. *Phys. E: Low-dimen Syst. Nanostruct*., https://doi.org/10.1016/j.physe.2019.113655

Das, K.K., Das, S.N., and Dhundasi, S.A., 2008. Ni, its adverse health effects and oxidative stress. *Indian J. Med. Res*., **128**: 412-425.

Dorofeeva, N.A., Tikhonov, D.B., Barygin, O.I., Tikhonova, T.B., Salnikov, Y.I., and Magazanik, L., 2005. Action of extracellular divalent cations on native α-amino-3-hydroxy- 5-methylisoxazole-4-propionate (AMPA) receptors. *J. Neurochem*., **95**: 1704–1712. https://doi.org/10.1111/j.1471-4159.2005.03533.x

Haber, L.T., Bates, H.K., Allen, B.C., Vincent, M.J., and Oller, A.R., 2017. Derivation of an oral toxicity reference value for Ni. *Reg. Toxicol. Pharmacol.,* **87**: S1–S18. https://doi.org/10.1016/j.yrtph.2017.03.011

He, M.D., Xu, S., Zhang, X., Wang, Y., Xiong, J.C., Zhang, X., Lua, Y.H., Zhang, L., Yua, Z.P., and Zhou, Z., 2013. Disturbance of aerobic metabolism accompanies neurobehavioral changes induced by Ni in mice. *Neuro Toxicol*., **38**: 9–16. https://doi.org/10.1016/j.neuro.2013.05.011

Hussain, M.F., Ashiq, M.N., Gulsher, M., Akbar, S., and Iqbal, F., 2020. Exposure to variable doses of Ni oxide nanoparticles disturbs serum biochemical parameters and oxidative stress biomarkers from vital organs of albino mice in a sex specific manner. *Biomark*, **25**: 719-724. https://doi.org/10.1080/1354750X.2020.1841829

Ijomone, O.M., Okori, S.O., Ijomone, O.K., and Ebokaiwe, A.P., 2018. Sub-acute Ni exposure impairs behavior, alters neuronal microarchitecture, and induces oxidative stress in rats' brain. *Drug Chem. Toxicol*., **41**: 377-384. https://doi.org/10.1080/01480545.2018.1437173

Iqbal, F., Muhl, A., Hoeger, H., and Bodamer, O.A., 2017. Biochemical and behavioral phenotype of AGAT and GAMT deficient mice following long-term creatine monohydrate supplementation. *Metabol. Brain Disord*., **32**: 1951-1961. https://doi.org/10.1007/s11011-017-0092-3

Khosa, T., Faiz, A., Haider, A., Ashiq, M.N., and Iqbal, F., 2020. Synthesis and characterization of newly synthesized neodymium zirconate zinc sulfide nano composite and its effect on selected aspects of albino mice behavior. *Naunyn-Schmiedeberg's Arch Pharmacol*., **393**: 717-725. https://doi.org/10.1007/s00210-019-01787-z

Lamtai, M., Chaibat, I., Ouakki, S., Zghari, O., Mesfioui, A., El Hessni, A., El-Housseine, R., Marmouzi, I., Essamri, A., and Ouichou, A., 2018. Effect of Chronic administration of Ni on affective and cognitive behavior in male and female rats: Possible implication of oxidative stress pathway. *Brain Sci*., **8**: 141-148. https://doi.org/10.3390/brainsci8080141

Liapi, C., Zarros, A., Theocharis, S., Voumvourakis, K., Anifantaki, F., Gkrouzman, E., Mellios, Z., Skandali, N., Al-Humadi, H., and Tsakiris, S., 2011. Short-term exposure to Ni alters the adult rat brain antioxidant status and the activities of crucial membrane-bound enzymes: Neuroprotection by L-Cysteine. *Biol. Trace Elem. Res*., **143**: 1673–1681. https://doi.org/10.1007/s12011-011-9006-0

Murthy, S.K., 2007. Nanoparticles in modern medicine: State of the art and future challenges. *Int. J. Nanomed*., **2**: 129–141.

Nation, J.R., Hare, M.F., Baker, D.M., Clark, D.E., and Bourgeois, A.E., 1985. Dietary administration of Ni: effects on behavior and metallothionein levels. *Physiol. Behav*., **34**: 349-353. https://doi.org/10.1016/0031-9384(85)90194-5

Oberdörster, G., Oberdörster, E., and Oberdörster, J., 2005. Nanotechnology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Hlth. Perspect*., **113**: 823–839. https://doi.org/10.1289/ehp.7339

Prendergast, D.M., Ardekani, B., Ikuta, T., John, M., Peters, B., and Derosse, P., 2015. Age and sex effects on corpus callosum morphology across the lifespan. *Hum. Brain Mapp.,* **36**: 2691–2702. https://doi.org/10.1002/hbm.22800

Rabelo, L.M., Costae, S.B., Almeida, S.F., Silva, W.A.M., Mendes, B.O., Guimarães, A.T.B., Silva, A.R., Castro, A.L.S., Rodrigues, A.S.L., and Malafaia, G., 2016. Memory deficit in Swiss mice exposed to tannery effluent. *Neurotoxicol. Teratol*., **55**: 45-49. https://doi.org/10.1016/j.ntt.2016.03.007

Raz, N., Gunning-Dixon, F., Head, D., Williamson, A., and Acker, J.D., 2001. Age and sex differences in

the cerebellum and the ventral pons: A prospective MR study of healthy adults. *Am. J. Neuroradiol*., **22**: 1161–1167.

Ren, G., Hu, D., Eileen, W., Cheng, C., and Miguel, A., 2009. Vargas-Reusc, Paul Reipd, Robert P. Allaker Characterisation of copper oxide nanoparticles for antimicrobial applications. *Int. J. Antimicrob. Agent*, **33**: 587–590. https://doi.org/10.1016/j.ijantimicag.2008.12.004

Sadiq, S., Ghazala, Z., Chowdhury, A., and Büsselberg, D., 2012. Metal toxicity at the synapse: Presynaptic, postsynaptic, and long-term effects. *J. Toxicol*., https://doi.org/10.1155/2012/132671

Siddiqui, M.A., Ahamed, M., Ahmad, J., Khan, M.M., Musarrat, J., Al-Khedhairy, A.A., and Alrokayan, S.A., 2012. Ni oxide nanoparticles induce cytotoxicity, oxidative stress and apoptosis in cultured human cells that is abrogated by the dietary antioxidant curcumin. *Fd. Chem. Toxicol*., **50**: 641-647. https://doi.org/10.1016/j.fct.2012.01.017

Song, X., Fiatikenston, S.S., Kong, L., and Zhao, J., 2017. Molecular mechanisms of Ni induced neurotoxicity and chemoprevention. *Toxicology*, https://doi.org/10.1016/j.tox.2017.10.006

Xin, J., Zhang, Y., Tang, Y., and Yang, Y., 2019. Brain differences between men and women: Evidence from deep learning. *Front. Neurosci*., **13**: https://doi.org/10.3389/fnins.2019.00185

Zaitseva, N., Zemlyanova, M., Akafeva, T., and Zvezdin, V., 2016. Assessment of potential hazard of Ni oxide nanoparticles. *Ekol. Cheloveka Hum. Ecol*., pp. 10-16. https://doi.org/10.33396/1728-0869-2016-10-10-16