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Absorption, LD50 and Effects of CoO, MgO and PbO Nanoparticles on Mice "*Mus musculus*"

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Abstract: *Metal oxide nanoparticles (NPs) are widely used in the world for different purposes. This is the first report on the LD50 and the effects of CoO, MgO and PbO NPs on mice. LD50 for acute and 30 day's chronic dosage of these metal oxide NPs was calculated. Our results showed that the acute (single dose exposure) LD50 for CoO is 7835 mg/kg, MgO is 3954 mg/kg and PbO is 14,677 mg/kg of body weight. Whereas, the LD50 for 30 days chronic dosage for CoO is 1066 mg/kg, MgO is 1315 mg/kg and PbO is 634.88 mg/kg of body weight. The NPs were deposited in different tissues of mice when exposed to acute and chronic dosages of all afore mentioned three different NPs. The NPs were maximally absorbed by the muscles on acute and chronic exposure to CoO, MgO as well as PbO NPs. The chronic exposure of CoO, PbO and MgO led to changes in behavior, body weight and organ index of the mice.*

Keywords*: Absorption, Cobalt oxide NPs; LD50; Lead oxide NPs; Magnesium oxide NPs, Organ Index.*

Abbreviations: *Nanoparticles (NPs), Cobalt oxide (CoO), Magnesium oxide (MgO), Lead oxide (PbO), body weight (bwt).*

I. Introduction

The nanoparticles (NPs) in the scale of 100 nm or less have numerous novel and useful applications in the field of electronics, chemical industry, environmental protection and bio-pharmacology. The nanosize particles are likely to increase unnecessary infinite toxicological effects on animals and environment, although their toxicological effects associated with human exposure are still unknown [1].

Metal NPs possess unique optical, magnetic and electrical activity [2]. Some magnetic NPs are proposed for medical uses such as drug delivery and hypothermic cancer treatments [3]. Magnetic NPs are also emerging as a class of novel contrasts agents for medicinal imaging [4] as in, when used for magnetic resonance imaging (MRI), they are very efficient as relaxation promoters, enhancing tissue contrast and helping to form sharper images of the area of interest [5].

Cobalt oxide NPs in particular are currently attracting enormous interest owing to their unique size- and shape-dependent properties and are used in pigments, catalyst, sensors, electrochemistry, magnetism, and energy storage devices [6, 7]. Iron oxide NPs are the most widely used contrast agent in MRI[8], but more recently cobalt NPs have been suggested as an alternative to iron due to their greater effects on proton relaxation[7,9]. Indiscriminate use of NPs like cobalt oxide could pose deleterious effects on the environment [10] and all the living organisms and possibly may enter the human body by ingestion, inhalation, and skin absorption [11]. Lead is used in metal work and in ceramics manufacture in Mexico and other countries and thus continues to be of concern to public health [12]. "Low-level" Pb toxicity (blood Pb level of 25-55 jug/dl) causes multiple metabolic, neurological and behavioral disorders. Many recent studies have indicated that even lower blood Pb levels (<25, ug/dl) are associated with impairment in mental development, decreased skeletal growth, and disturbances in cardiovascular function [13]. MgO NPs are well known for their biological applications as an antibacterial agent, for the relief of heart burn and bone regeneration. MgO is one of the six magnesium compounds which are currently recognized as safe by the U.S. Food and Drug Administration (21CFR184.1431). Earlier reports reveal the cytotoxicity of MgO NPs towards human umbilical vein endothelial cells and on human cardiac microvascular endothelial cells [14].

NPs can also enter the host systems via skin spores, debilitated tissues, injection, olfactory, respiratory and intestinal tracts. These uptake routes of NPs may be intentional or unintentional. Their entry may lead to diverse adverse biological effects. Until a clearer picture emerges, the limited available data suggest that a caution has to be exercised when potential exposures to NPs are encountered. Methods used in determining NPs ports of entry into experimental animals include pharyngeal instillation, injection, inhalation, cell culture lines and gavage exposures [1]. So far, most of the nanotoxicity research focused on respiratory tract exposures for assessing the health effects of NPs. Other exposure routes e.g., gastrointestinal tract also needs to be considered as potential ports of entry. There are many ways that NPs can be ingested into the gastrointestinal tract. For instance, NPs cleared from the respiratory tract via the mucociliary escalator are likely to enter into the gastrointestinal tract; NPs could be ingested directly via water, food, cosmetics, drugs, drug delivery devices,

etc. Uptake of particles of different size via gastrointestinal tract can be toxic. However, reports of toxicity of NPs due to their absorption by the gastrointestinal tract are very few [15].

The data on the effect of NPs is very much limited so it is very important to focus on this area. In our study we have tried to evaluate the effects of few NPs like CoO, MgO and PbO administration by oral/ gastrointestinal route.

II. Materials And Methods

Metal oxide NPs such as, CoO (average size 56.57 nm), PbO (average size 48.87nm) and MgO (average size 26.35 nm) were prepared using chemical precipitation method reported by Yazid to prepare Zinc Oxide [16]. All the chemicals used were of analytical grade.

Animal model: Mice (Mus musculus)

Maintenance of Animals:

The Swiss Albino Mice (Mus musculus), weighing 20-35 grams were housed in polypropylene cages and were maintained at ambient laboratory conditions(temp.=22-24^{0}C, humidity=75%, dark-light cycle=14/10 with free access to water) and standard pellet diet (Hindustan Lever, Bangalore, India), Ethical approval was obtained from the Institutional Animal Ethics Committee (Ref no. 206/C -2007), based on the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines[17], which were followed throughout the study. The animals were maintained at animal house facility of the Department of Zoology, Goa University.

LD50 measurement for Acute and Chronic Toxicity

To obtain the LD50 of NPs, the experiments and its intervals were designed in accordance with the method provided by the Organization for Economic Cooperation and Development [18]. The first animal receives a dose one step below the assumed estimate of the LD50. If the animal survives, the second animal receives a higher dose. If the first animal dies, the second animal receives a lower dose [15]. Further the probit analysis was performed and the Ld50 was calculated [19].

Acute toxicity: The animals were divided into groups for acute exposure. They were gavaged with single dose of NPs such as PbO, CoO and MgO and monitored 72 hrs for their behavior, motor impairment and survival. For each dose the mice were divided into 5 groups with each group having 10 mice and fed with different concentrations of PbO, CoO and MgO ranging from low to high (2000 to 17000, 1000 to 10000 and 1000 to 5000 mg/kg bwt respectively). The symptoms were monitored and the LD50 was calculated using probit analysis [19, 20].

Chronic toxicity: The animals were divided into groups for 30 days exposure. The mice were gavaged with NPs such as PbO, CoO and MgO continuously for a period of 30 days and monitored for their behavior, motor impairment and survival. For each dose the mice were divided into 5 groups, each group having 10 mice and were fed with different concentrations of NPs(PbO, CoO, MgO) ranging from low to high(200 to 1200 , 800 to 1800, 800 to 1800 respectively). The symptoms were monitored and the LD50 was calculated using probit analysis [19, 20].

Deposition of NPS in the body: The mice were divided into 6 groups, 3 groups for acute exposure and 3 groups for 30 days chronic exposure of NPs. Each group had 6 mice. Based on the Ld50 values the sub lethal doses were selected for exposure. For acute exposure the mice were fed with PbO (25mg/kg bwt), MgO (25mg/kg bwt) and CoO (25mg/kg bwt). For 30 days exposure the mice were fed with PbO (6mg/kg bwt), MgO (12mg/kg bwt) and CoO (10mg/kg bwt). Further, the mice were sacrificed by decapitation, the tissues (heart, brain, kidney, muscles, liver, intestine, spleen, pancreas) were removed and rinsed with mammalian saline and stored at -4^{0}C until use. Tissue samples were then dried completely in a clean oven at 60–70^0 C. Each dried tissue was digested to white ash using 30% hydrogen peroxide at 50–60^0 C followed by digestion with concentrated 0.1mL of ultra-pure trace-metal free nitric acid. The digested white ash was dissolved in 0.25 N nitric acid (1–5 mL) based on the weight of the digested tissue. Each digested sample was further diluted suitably (1:2–1:50 v/v, depending on the tissue type and amount) using 0.25 N ultra-pure nitric acid prior to analysis [21]. The analysis was performed using model No-Varian AA 240 FS with Air acetylene flame. Standards (Sigma Aldrich Chemical Co., Milwaukee, WI) were used for all calibration curves and for verifying metal recovery from tissues Bovine Liver standard samples were used (1577b, U.S NIST Gaithersburg, MD 20899).

Organ Index: To examine the grade changes caused by these nanoparticles organ index was carried out for Brain, Spleen [15], Kidney, Liver, Heart and Pancreas. The organ index is defined as:

$$\text{Organ Index} = \frac{\text{Weight of Experimental organ} \div \text{weight of the experimental mice}}{\text{Weight of control organ} \div \text{weight of the control mice}}$$

General examination: After administration of NPs, animals were examined daily for their survival, evident behavior or motor impairments and effect on their body weight.

Behavioral changes: like drowsiness, hyperactivity, moving of tail, scratching of body, loss of hair, texture of hair (smooth, or rough) etc were observed**.**

Body weight: The change in body weight was recorded after every 6 hours for acute exposure and after every 24 hours for 30 days exposure for three different concentrations.

Statistics: Statistical analyses were done using XLSTAT software. Data were expressed as means ± SE. Student t- test was performed to compare the differences of means among two groups and Two-way analysis of variance (ANOVA) was carried out to compare the differences of means among multi-group data. The difference was considered significant at $P \leq 0.05$ and highly significant at $P \leq 0.001$.

III. Results

LD50: After performing the probit analysis it was found that the LD50 of CoO, MgO and PbO for acute toxicity was **7.835 g/kg** , **3.954 mg/kg** and is **14.677 g/kg** of body weight respectively(Fig 1). Whereas, the LD50 for 30 days chronic dosage for the same NPs was **1.066 g/kg, 1.315 g/kg** and **0.63488 g/kg** of body weight respectively(Fig 2). At acute exposure CoO and PbO NPs were practically non toxic (Class 5) as the Ld50 more than 5000mg/kg is considered to be nontoxic and MgO is slightly toxic (class 4) by Hodge and sterner scale [22], whereas CoO and PbO NPs were slightly toxic and MgO was moderately toxic by Gosselin, Smith and Hodge scale [23]. At sub chronic exposure all the three NPs were seen to be slightly toxic (class4) by Hodge and sterner scale and moderately toxic by Gosselin, Smith and Hodge scale.

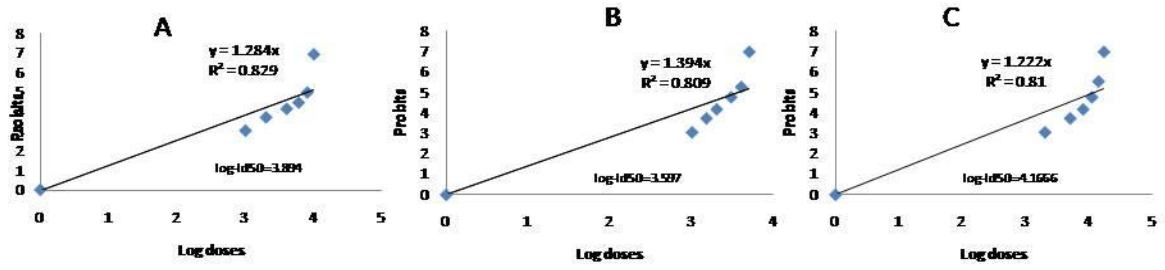

Fig 1: Acute LD50 after single dose exposure to (A) CoO NPs (B) MgO NPs and (C) PbO NPs

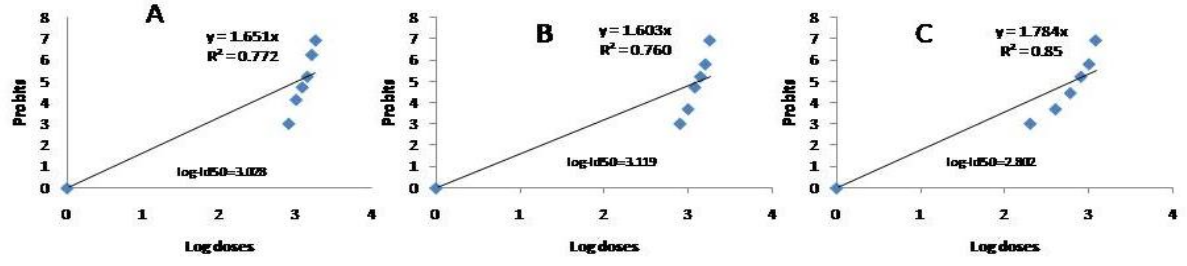

Fig 2: Sub-chronic LD50 after continuous 30 days exposure to (A) CoO NPs (B) MgO NPs and (C) PbO NPs

Absorption of NPs: The absorption of NPs was observed in most of the vital organs of the body. The recovery of the metals from the standard tissue sample (Bovine liver 1577b) was in the range of 98-99%. The maximum absorption was seen in muscles for CoO, PbO and MgO NPs both for acute and chronic exposure followed by intestine. The least absorption was observed in brain for all three NPs for acute as well as chronic treatment

(Table 1-3). The average value of organ index in all the tissues showed variations when compared with normal parameter obtained from the control mice (Table 4).

Table 1: Deposition of Cobalt oxide NPs after acute (single dose exposure) and sub chronic (30 days continuous exposure) to different parts of the body. (All the values are expressed as means ± SE).

Deposition of CoO NPs						
Organ	Acute O.D	Acute Conc. in µg/gm dry wt. of tissue	Acute % distribution in the body	Chronic O.D	Chronic Conc. in µg/gm dry wt. of tissue	Chronic % distribution in the body
Brain	0.024	2.63 ± 0.097	0.112	0.054	7.71 ± 0.751	0.387
Heart	0.076	15.1 ± 0.176	2.276	0.103	17.16 ± 0.266	3.045
pancreas	0.086	21.6 ± 0.220	3.69	0.035	9.73 ± 0.138	1.966
Liver	0.048	4.80 ± 0.057	5.59	0.067	7.41 ± 1.291	10.19
Muscles	0.053	10.6 ± 0.115	67.75	0.064	8.08 ± 0.220	60.93
kidney	0.052	7.38 ± 0.125	2.127	0.065	8.16 ± 0.222	2.776
Intestine	0.033	6.60 ± 0.115	17.37	0.029	6.44 ± 0.359	20.00
Spleen	0.051	12.8 ± 0.083	1.072	0.067	6.98 ± 0.359	0.687

Table 2: Deposition of Magnesium Oxide NPs after acute (single dose exposure) and sub chronic (30 days continuous exposure) to different parts of the body. (All the values are expressed as means ± SE).

Deposition of MgO NPs						
Organ	Acute O.D	Acute Conc. in µg/dry wt. of tissue	Acute Percentage	Chronic O.D	Chronic Conc. in µg/dry wt. of tissue	Chronic Percentage
Brain	0.315	98.635 ± 0.275	0.315	0.610	142.96 ± 5.757	0.580
Heart	0.230	143.75 ± 3.662	1.726	0.335	123.80 ± 15.57	1.884
pancreas	0.272	113.58 ± 1.463	1.434	0.327	149.40 ± 4.167	2.390
Liver	0.249	88.966 ± 0.178	7.753	0.394	133.12 ± 16.15	14.70
Muscles	0.336	140.02 ± 1.403	66.83	0.301	84.70 ± 7.099	51.24
kidney	0.606	126.41 ± 0.012	2.686	0.429	80.94 ± 4.651	2.180
Intestine	0.294	92.041 ± 0.745	18.42	0.335	102.5 ± 20.84	26.01
Spleen	0.203	127.16 ± 0.126	0.822	0.265	122.39 ± 9.631	1.003

Table 3: Deposition of Lead Oxide NPs after acute (single dose exposure) and sub chronic (30 days continuous exposure) to different parts of the body. (All the values are expressed as means ± SE)

Deposition of PbO NPs						
Organ	Acute O.D	Acute Conc. in µg/dry wt. of tissue	Acute Percentage	Chronic O.D	Chronic Conc. in µg/dry wt. of tissue	Chronic Percentage
Brain	0.164	27.33 ± 0.192	0.336	0.189	34.879 ± 2.521	0.335
Heart	0.218	31.23 ± 0.629	1.453	0.144	18.041 ± 2.361	0.654
pancreas	0.192	48.00 ± 0.144	2.655	0.207	51.916 ± 3.001	2.240
Liver	0.207	18.81 ± 0.104	6.671	0.273	31.941 ± 2.876	8.834
Muscles	0.293	36.70 ± 0.291	70.60	0.324	46.733 ± 3.844	70.125
kidney	0.314	28.54 ± 0.240	2.619	0.281	28.166 ± 1.922	12.016
Intestine	0.218	16.82 ± 0.285	13.95	0.164	22.745 ± 1.429	14.723
Spleen	0.176	58.77 ± 0.484	1.699	0.244	47.444 ± 6.532	1.070

Table 4: Organ index of mice exposed to CoO, MgO and PbO after sub chronic (30 days continuous exposure) to different parts of the body. (All the values are expressed as means ± SE)

Organ Index	Control	CoO NPs	MgO NPs	PbO NPs
Brain index(Bx) in g/bwt	1.646±0.249	2.135±0.232	0.932±0.020	1.285±0.039
Spleen Index(Sx) in g/bwt	0.647±0.100	1.699±0.027	0.482±0.142	0.680±0.038
Kidney Index(Kx) in g/bwt	1.233±0.030	2.381±0.064	0.586±0.017	1.351±0.054
Liver Index(Lx) in g/bwt	5.451±0.128	1.630±0.023	0.415±0.038	1.065±0.036
Heart Index(Hx) in g/bwt	0.450±0.004	2.189±0.119	0.862±0.125	1.142±0.086
Pancreas Index(Px) in g/bwt	0.538±0.026	3.192±0.361	0.488±0.012	0.121±0.007

General examination: The mice exposed to MgO NPs showed hyperactivity, moving of tail but the hair remained smooth throughout the exposure and those exposed to PbO NPs showed hyperactivity initially for 6-7 days but further showed drowsiness and the hairs were semi smooth. Whereas the mice exposed to CoO NPs showed hyperactivity for 2-3 days followed by drowsiness, scratching of body, loss and roughness of hair and also skin color started changing to bluish black after 10 days of exposure and was darker by the end of the month.

Body weight: Mice exposed to acute single doses of all three NPs separately did not show any effect on the body weight as compared to controls. Whereas when the mice exposed for 30 days to MgO showed significant (P>0.001) increase in their body weights (Fig 4) whereas, the weight of mice exposed to CoO and PbO NPs decreased significantly (P>0.001) (Fig 3 and 5). It was also noted that the effects were dose dependant and time dependant; however, both the time and concentrations factors were effective independently.

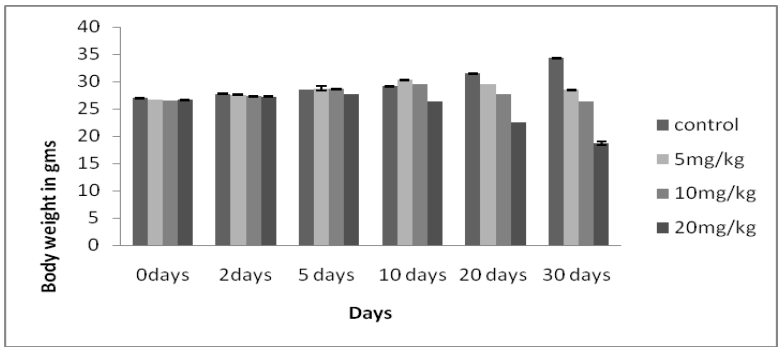

Fig 3: Effect of different concentrations of cobalt oxide NPs on the body weight of mice (P≤0.001)

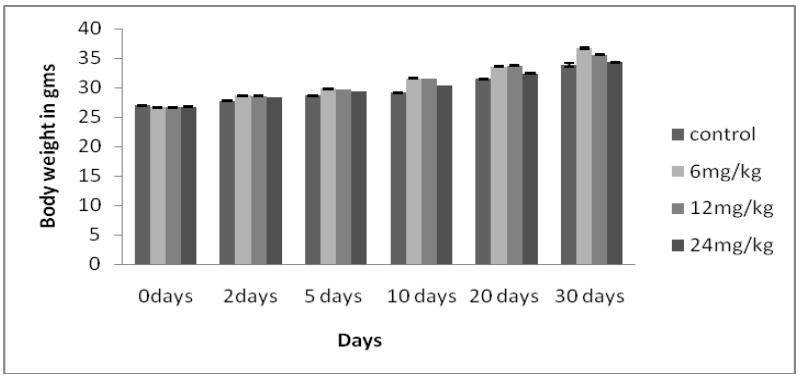

Fig 4: Effect of different concentrations of Magnesium oxide NPs on the body weight of mice (P≤0.001)

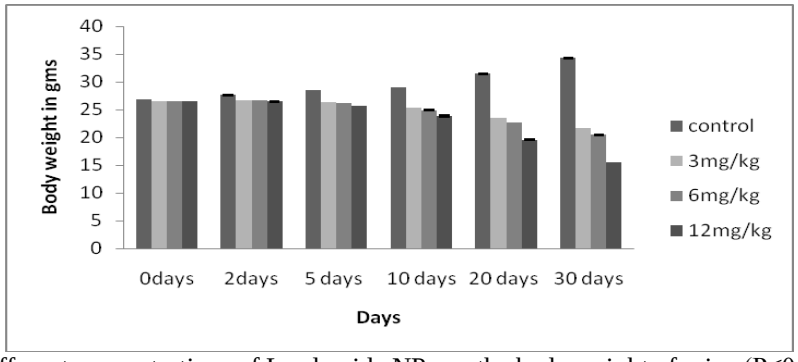

Fig 5: Effect of different concentrations of Lead oxide NPs on the body weight of mice (P≤0.001)

IV. Discussion

The toxicity of few metal oxide NPs (CoO, MgO and PbO) to mice by gastrointestinal tract exposure was studied. This is a first report of the LD50 of CoO, MgO and PbO NPs. The ld50 value of non nano metal salts of Pb (Lead acetate), Co (Cobalt acetate) and Mg (Magnesium sulphate) are reported to be 4.665 g/kg, 0.503g/kg and 5g/kg bwt respectively [24-26]. These clearly indicates that this metals viz; Co and Pb in nanoform are relatively less toxic in acute and chronic exposures but Mg appears to be more toxic then metal salt. CoO and MgO belong to toxicity Class 5(practically nontoxic) and PbO to Class 4 (slightly toxic) of Hodge and sterner scale[22], but according to Gosselin, Smith and Hodge scale[23] CoO and MgO belong to toxicity Class 4(slightly toxic) and PbO to Class 3 (moderately toxic) at acute exposure. For chronic exposure the toxicity classes for all 3 NPs are Class 4 (slightly toxic) of Hodge and sterner scale but according to Gosselin, Smith and Hodge scale, it belongs to toxicity Class 3(moderately toxic) [22,23]. In the present study it was noticed that the NPs at single dose (acute) exposures were nontoxic or slightly toxic but with the continuous(sub chronic) exposure the same NPs were highly toxic to the body. Lead as a metal is considered to be highly toxic, while

cobalt is believed to be toxic but magnesium is said to be nontoxic or least toxic. However, the present work indicates that a nanosize magnesium oxide was most toxic and lead oxide was least toxic at acute exposure, suggesting that the properties of the metals and its effects or toxicity changes with the size of the substance. As the size of the particles decrease, the reactivity increases and normally harmless substances may cause hazardous effects and harmful effects may be intensified and vice-versa [27].

It was found during the acute treatments that muscles absorb more NPs as compared with other tissues and this could be attributed to muscles occupying largest mass in the body [28]. Similar results were found for the sub-chronic treatment of CoO, PbO and MgO NPs. The intestine was the next after muscles to absorb most of the afore mentioned NPs as these NPs might have stuck to the intestinal lining or intestinal tract or retained due to their quick adsorbability to the protein moieties or carrier proteins of the membrane leading to limited transport to the other tissues of the body. Brain receives the least NPs as most of the NPs are absorbed by the intestine and muscles on the way but this indicates that blood brain barrier is either compromised or these NPs can cross such barrier. Viswakarma et al and Wang et al[29,30] are of view that small amount of NPs are able to travel towards the brain and reach the brain circulation and out of that very few are able to pass through blood brain barrier and enter the brain. Buzea et al [31] have suggested agglomeration of NPs owing to their magnetic property and such agglomerates/clusters prevent transport of NPs across the blood brain barrier. Further the organ index was examined to observe the grade of changes caused by these nanoparticles. It was noticed that acute exposure did not show any change in organ index as the nanoparticles must be eliminated out from the body, as well as exposure time was less to cause any damage to the organs, whereas at the sub-chronic exposure because of the repeated administration of NPs for 30 days the entire organ index increased in mice exposed to CoO NPs except the liver. Mice exposed to MgO showed decrease in all organ indexes except Heart. Whereas Brain, Kidney, Heart index increased and Liver, Pancreas index decreased in mice exposed to PbO NPs. The spleen index did not show much change in comparison to control. The increased organ index attributes to inflammation and decrease to degeneration of tissue or atrophy [15] of the organs caused by these nanoparticles.

CoO, MgO and PbO NPs at single dose exposure did not affect the body weight of mice when compared with controls as most of the NPs might have been excreted out so that they were not absorbed by the tissue to a threshold level to produce a toxic effect. When mice were exposed for 30 days to the NPs they could have gradually reached an effective threshold level. Chronic exposure of mice to MgO NPs caused significant increase in their body weight. The increase in the body weight of mice could be due to hormonal imbalance promoted by MgO NPs and any type of stress to the body causes excess secretion of stress hormone such as cortisol which in turn increases body fat [32]. However, it needs further investigation to throw more light on this aspect. Further, the mice exposed to CoO and PbO NPs showed significant decrease in their body weight. Decrease in weight might be due to loss of appetite and less intake of food material. Since the mice exposed to CoO and PbO NPs were drowsy and dull, their food intake was reduced as compared to the controls.

In the present study, the mice showed many signs of behavioral alterations like induction of hyperactivity after administration of NPs particularly PbO and CoO followed by drowsiness. The initial reactions may be due to sudden intake of foreign particles [31]. The CoO NPs may have been carried by the blood to different tissues and this led to their deposition. The change in skin color could be attributed to the deposition of NPs which are black in color and also partly owing to their interaction with the membrane proteins. The hair texture also became rough when exposed to CoO and PbO NPs as the NPs carried by the blood gets deposited in the skin (confirmed by AAS) and are further transferred to the hair follicles thereby affecting the keratin which could change the texture of the hair. Further the mice dosed with MgO showed hyperactivity and moving of tail. The MgO nanoparticles on entering the brain (confirmed by AAS) could be decreasing effective intracellular traffecking protein called LMTK3 mostly present in two brain regions cerebral cortex and hippocampus, the cerebral cortex coordinates perception, movement, and thought, and the hippocampus, governs memory and learning, so lack of LMTK3 in these brain regions causes hyperactivity and moving of tail in mice [33].

The increased industrial usage and the applications of CoO, MgO and PbO NPs in different fields can increase the chances of its exposure to the animals and human beings and also its release in the environment. Our study determines the LD50 of the three metal oxide NPs (CoO, MgO and PbO). We have also reported that these NPs are absorbed/ retained in all the organs of the body affecting the behavior and body weight of the animal, thus providing its potential effect on the normal functioning of the animal as well as the human being.

V. Conclusion

The LD50 of the three metal oxide NPs (CoO, MgO and PbO) is been reported. The absorption/retention of NPs in the body, change in body weight and the behavioral changes after exposure to these NPs show that CoO, MgO and PbO NPs are toxic and affecting the tissues/organs of the body. The acute exposure did not show much change in behavior and organ index after deposition of NPs but the sub-chronic exposure appears to influence the behavior and internally affect the organs of the mice after deposition of the NPs.

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