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Histotoxicity induced by copper oxide nanoparticles (CuO-NPs) on developing mice (*Mus musculus*)

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ABSTRACT

The wide range of applications of nanoparticles (NPs) in various industries have led to serious consequences in terms of teratogenic toxicity. The aim of current work was to evaluate the teratogenic effects of copper oxide (CuO) nanoparticles in albino mice.In this experimental study, after mating, inseminated 40 female mice were divided randomly into 4 pools (1 control and 3 experimental), ten each. Doses were administered intravenously (We followed the protocol by Yaqub et al. (2018), intravenous application is faster route as compared to oral dosage)to all the experimental groups on the 6th day of gestation (GD), dose concentrations were 200, 133.3 and 100 mg/kg body weights respectively.The doses were prepared in sequence (1/2, 1/3, 1/4 0f LD50) according to already published work. The effects of CuO-NPs show linear relationship with the above sequence. The control group was administered only with distilled water.The gravid females were sacrificed through cervical disruption at the 18th day of gestation, fetuses were removed and divided into four sets (pools) for morphometric, morphological and histological studies. Data were subjected to statistical analysis by using Tukey's test in light of ANOVA at $p < 0.05$ level of significance. Findings of the present study showed that CuO-NPs various concentrations affect developmental abnormalities i.e.runt embryos, resorbed uteri, exencephaly, hygroma, macroglossia, micromelia, open eye, omphalocoel, scoliosis, kyphosis and kinked tail. It is concluded that exposure to CuO-NPs may potentially lead to the developmental deformities in mice.

1. Introduction

The world is rapidly shifting to nanotechnology. A product of nanotechnology is NPs. These particles have their size in any dimension (height, length and width) between 1 and 100 nm (nm) (Madkour, 2019). The nanostructures are synthesized and manufactured on large scales having exceptional physicochemical characteristics. Moreover, the size of nanostructures and their physicochemical properties changed accordingly (Zhang et al., 2014; Siddiqui et al., 2020). This work is on CuO-NPs having size of 50 nm. These are in black colored powder (Kamal, 2019).CuO-NPs has many features (photonic properties, electronic and optical) for this reason it is used in different commercial applications widely like catalysts, gas sensors, photovoltaic cells, thermal shift Nano fluids, antibacterial and antifungal agents. Additionally, CuO-NPs are utilized as additives in lubricants, polymers and metallic coatings (Mallakpour et al., 2020; Madkour, 2018; Singh, 2019).NPshas a magical potential; it can revolutionize the agricultural industry. Important fields where it has already worked wonders are food processing, packaging and preservation (Panzade,2010; Sohniet al., 2018; Rajput et al., 2018). CuO-NPs have large surface area to volume ratio so act as a potent antimicrobial agent (Applerotet al., 2012; Guet al., 2018). These enable us to save our food from various bacterial and fungal diseases and also help us store food without causing any disease for a long time (Rajput et al., 2020; Guet al., 2018; Tharchanaaet al., 2021). CuO-NPs have a lot of benefits and applications but have many menace and toxic effects on animal and human life. Researchers have evaluated the possible toxicity and harms of NPs on humans and found that the toxicity is directly proportional to the size (Yaqubet al., 2018; Wongrakpanichet al., 2016; Yusefi-Tanhaet al., 2020). The NPs exposure route to humans may also be an important factor in inducing toxicity, involving different mechanisms (Chang et al., 2012; Katsumitiet al., 2018). These may cause lethal effects like hemorrhage (Yaqubet al., 2018), cold sweating (Wang et al., 2018), headache

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Labels

3rd V	third ventricle
AO	Anophthalmia
Ao	Aortic arc
At	Atria
BC	Bicuspid valve
BF	Brown fat
BW	Body Weight
Com	Compressed
CuO-NPs	Copper Oxide Nanoparticles
D	Drooped
DC	distended chest
E	Eye
Ex	Exencephaly
Fib	Fibula
FL	Forelimb
GD	day of gestation
H	Head
Hm	Hemorrhage
HL	Hind limb
Hu	Humerus
Hv	Heart ventricle
Hy	Hygroma
I	Intestine
KT	Kinked tail
KY	Kyphosis
L	lung
Li	Liver
LV	Lateral ventricle
Md	Mandibular gland
Mg	Macroglossia
MI	Micromelia
Mo	Molar
MS	Misshapen
NBC	National Bioethics Committee
NE	Nasal epithelium
nm	nanometers
OE	Open eye
Om	Omphalocoe
lPG	pelvic girdle
R	ribs
R	Runt
RU	Resorbed uterus
S	Snout
S	sternum
SC	spinal cord
Sc	Scoliosis
Sk	skull
ST	Short tail
St	Stomach
T	Tail
Th	Thyms
Tib	Tibia
UB	Urinary bladder

(Egorova and Ananikov, 2017), disorders of kidneys (Hong et al., 2016; Yaqubet al., 2018) and nervous system (Sawickiet al., 2019; Maisanoet al., 2015). These may cause demyelination of neurons and nerves (Mohamed Mowafy et al., 2021; Carroet al., 2019; Maisanoet al., 2015). A research by Abudayyaket al., 2016; Ding et al. (2015) observed that the CuO-NPs encourage rigorous damage to murine kidney, spleen and liver. CuO-NPs increase the liberation of free radicals leading to hepatotoxicity and nephrotoxicity. CuO-NPs accumulate in cornea and bring about symptoms akin to Wilson's disease (Xu et al., 2017; Cohen et al., 2013). In regards to hematological effects, these cause hemolytic anemia and aggravates arteriosclerosis (Sharma et al., 2020). Furthermore, exposure to CuO-NPs leads to failure in mitochondrial function (Gallo et al., 2018).

The study of developmental abnormalities including the mechanisms and pattern of anomalous development is termed teratology (Ujházyet al., 2012). At the gastrulation and neurulation stages the mice embryo is vulnerable for teratogens to induce changes in development (Lipinski et al., 2010). So, any intrusion in the normal gastrulation and neurulation processes, following anomalous or abnormal congenital development (Ujházyet al., 2012). The previous exposure of CuO-NPs at these stages will not be studied properly.

Keeping in view the above and more toxicological studies, it is need of the hour that first, we should measure toxic and harmful impacts of CuO-NPs and then cope with toxic effects of it. After having coped with toxic impacts, it should permit its use.

2. Materials and methods

2.1. Copperoxide nano-particles (CuO-NPs)

The CuO-NPs (analytical grade)were obtained by Sigma-Aldrich (Chemie GmbH Eschenstrasse) Company with Product Number: 544868, REACH No. 01-2119502447-44, EC-No. 215-269-1 and CAS-No. 1317-38-0 having molecular weight 79.55 g/mol.

2.2. CuO-NPs characterization

2.2.1. XRD

The crystalline phase analysis of the synthesized CuO-NPs was determined by X-ray diffraction (XRD) (Fig. 1). The XRD peaks were observed at 38.21°, 44.30°, 64.52° and 77.47° (2θ values) which correspond to 110, 002, 111 and 202 lattice planes of face centered cubic silver (JCPDS file 04–0784). The peak intensity at 111 is greater than the other planes. These 111 facets were found to be the most reactive due to the high atomic density.

2.2.2. SEM analysis

The surface topography of newly synthesized CuO-NPs is given in Fig. 2. The SEM results showed that the surface is highly active and porous. Due to their highly porous structure, CuO-NPs have high absorption properties, which helped the adsorption of water-soluble dyes.

2.2.3. Histogram of CuO-NPs

The Histogram date was obtained from SEM and HRTEM micrographs, and the average particle size was analyzed using ImagJ software. Fig. 3 showed the small and narrow size distribution of CuO-NPs in the range of 0.9–60 and the average particle size is about 42 nm.

2.3. Animals

All in Vivo studies were on the Swiss Webster strain of albino mice (*Mus musculus*). 48 healthy mice (8 males 40 females), having average weight of 30 g were purchased from the animal house of the department of zoology, University of Veterinary and Animal Sciences, Lahore, Pakistan. The mice were kept in the mouse specific steel cages with dimensions of 12″ × 18″. To acclimatize the animals, these cages were placed in properly cleaned and ventilated rooms by maintaining relative humidity 60–65%, temperature 26 °C–30 °C and 12 h dark and light cycle for a week. This work was carried out in accordance with the

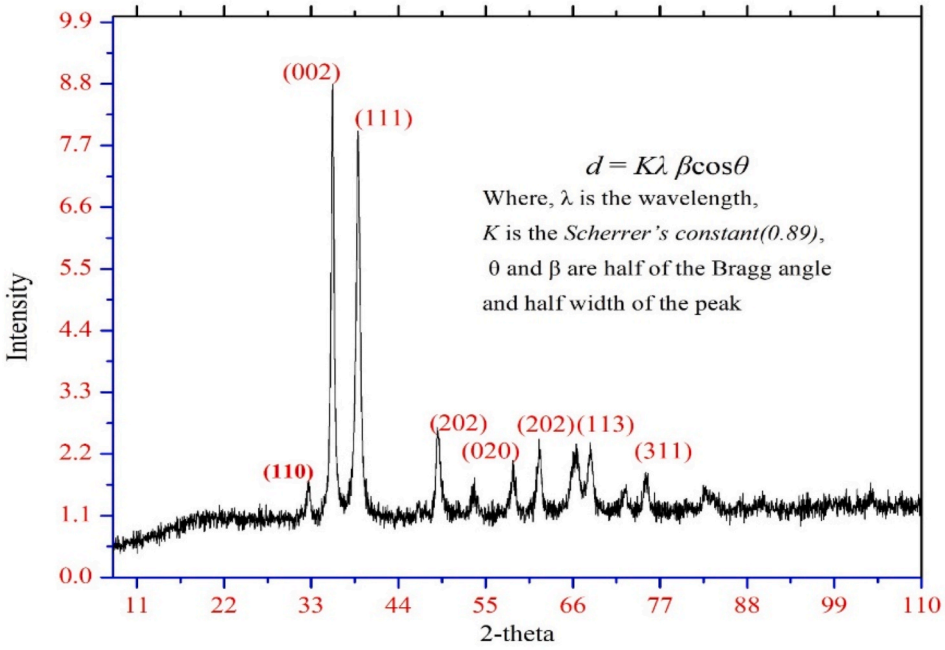

Fig. 1. XRD powder pattern for the CuO nanoparticles (NPs).

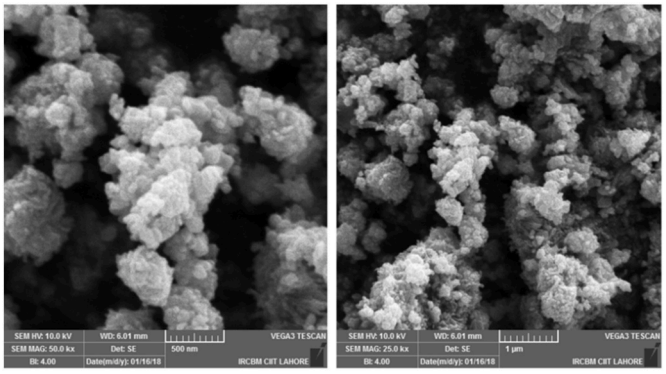

Fig. 2. (a) and (b) showing the ESEM images of copper oxide nanoparticles (CuONPs) at 50000 and 25000 magnification.

guidelines of the National Bioethics Committee (NBC) of Pakistan. All the mice were fed on standard commercial animal food and were provided with clean water ad libitum. This work was done in the Department of Zoology, University of the Punjab, Lahore, Pakistan.

2.4. Design of the experiment

After mating, inseminated 40 female mice were divided randomly into 4 pools (1 control and 3 experimental), ten each.

The control group was administered only the distilled water, whereas a single dose (D) of the CuO-NPs different concentrations was given intravenously to the experimental groups. ½, 1/3 and ¼ of CuO-NPs LD50 were used as three doses and the concentrations were 200 mg/kg BW (D1), 133.3 mg/kg BW (D2) and 100 mg/kg BW (D3) respectively (Kadammattilet al., 2018). The LD50 value for copper oxide nanoparticles (CuO-NPs) was not directly mentioned in our paper. The LD50 value utilized in our study was obtained from a previously published source, specifically Yaqub et al. (2018). The experimental conditions for our study are as follows: The Animal Species was Swiss Webster strain of albino mice (Mus musculus). And the Route of Administration:CuO-NPs were administered intravenously to the experimental groups. The doses were given on the 6th day of gestation via intravenous injection. The control group received distilled water.

2.5. Dose administration

The zero day of gestation was determined by the observing deposition of the semen or the vaginal plug after fertilization (we conducted mating between female and male mice as the method of fertilization. Artificial insemination was not employed)(Dewar, 1968). The stock solutions were prepared in such a way that 0.1 ml contained required dose and injected intravenously (Ashajyothi and Handral, 2018) with the help of needle (30G) to experimental groups. The protocol used in this work is from Ahmad et al. (2022).The doses were given at the 6th day of gestation. (Geraetset al.,2014).The choice of a single dose for our study, based on sub-lethal concentrations derived from the LD50 value, was made with the safety and well-being of the animals in mind (Ahmad et al., 2022; Fraga et al., 2014). The mortality rate was zero throughout the experimental trial.

2.6. Fetuses collection

At the 18th day of gestation the gravid females were humanely anesthetized with sedating ether and sacrificed through cervical disruption. The decision to sacrifice the mice on day 18 (D18) was made based on research objectives and considerations. While OECD guidelines recommend day 20 for teratology studies, D18 was chosen to potentially capture early developmental effects, as organogenesis is typically completed by this stage. Then uteri were dismembered through surgical incision, fetuses were removed and divided into four sets (pools). For further morphological, skeletal histological and morphometric studies fetuses were carefully weighed and were subjected to further processing.

2.7. Morphological observations

A set number of fetuses were placed in Bouin‟s fixative for 48 h, then transferred to 70% ethanol. The fetuses were sensibly analyzed for morphological anomalies under stereoscopic binocular microscope (Labomed Ltd Japan). The fetuses having any abnormalities were counted and docketed. The morphologically abnormal fetuses were

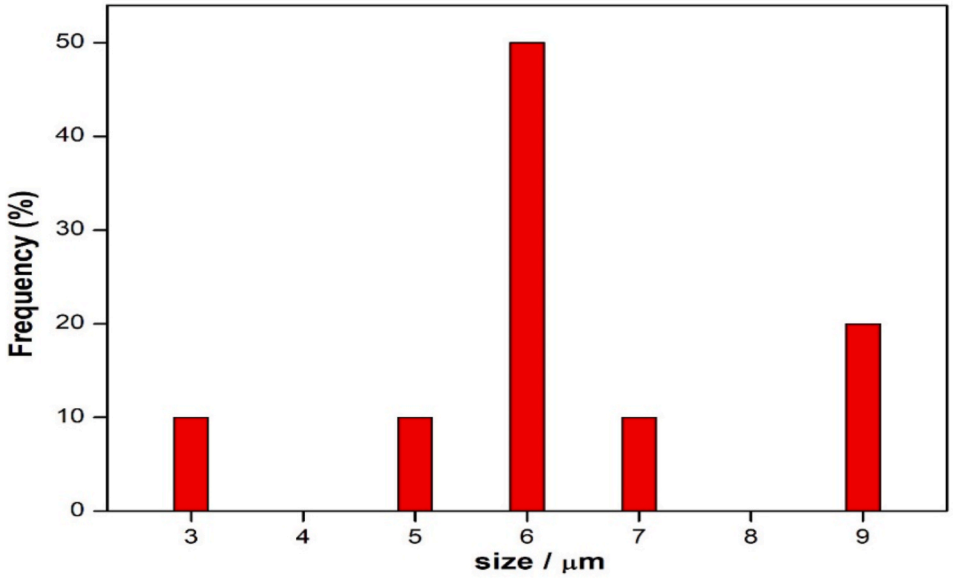

Fig. 3. Histogram illustrating the distribution of particle sizes for CuO-NPs.

captured by using a digital camera (Panasonic TZ15).

2.8. Morphometric analysis

Every fetus was further observed prudently for morphometric studies. The fetal weight, crown rump length, head circumference, eye circumference, length of fore-limbs, hind-limbs and tail were measured.

2.9. Histological preparations

Four fetuses were selected randomly from each group including control and experimental for histological studies. Fetuses were prepared by following the histological preparation techniques given by Scudamore (2014).

Micro-anatomical anomalies were observed under stereoscopic compound microscopy using atlas of mouse development (Kaufman, 2008). Histological photographs were taken by using a Nikon D90 camera.

2.10. Fetal skeleton preparation

Some of the fetuses were placed in 95% alcohol for skeleton preparations. Kawamura et al.1990 protocol was used to prepare skeletons for each group. A camera-fitted microscope (Labomed LX 200) was used for taking the microphotographs of stained skeletons.

2.11. Statistical analyses

Data were subjected to statistical analysis employing Tukey's test in ANOVA at $p < 0.05$ (level of significance) using SPSS (Statistical Package for the Social Sciences, version 20.0).

3. Results

3.1. Growth and development

Control group show normal growth and development (Fig. 4) whereas a single lowest dose (100 mg/kg BW) of CuO-NPs displays fetuses with anomalies like distended chest, exencephaly, hemorrhages, Kinked and small tail, micromelia shown in (Fig. 5).A single intravenous injection of medium dose (133.3 mg/kg BW) produces anomalies like hemorrhage, open eye, scoliosis, short tail, hygroma and runt embryos

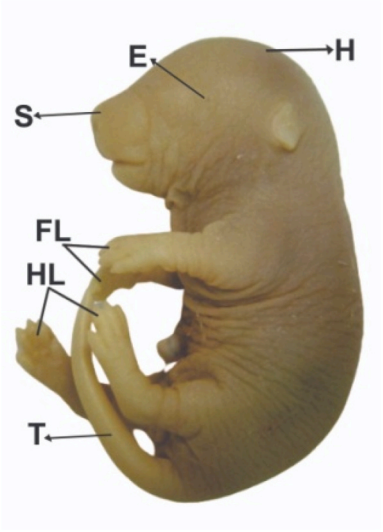

Fig. 4. Macro-photograph of 18 days old fetuses recovered from untreated dams.
Labels: E: Eye, FL: Forelimb, H: Head, HL: Hind limb, S: Snout, T: Tail.

as shown in Fig. 6.High dose group (200 mg/kg BW) giving single CuO-NPs dose intravenously, anomalies like dropped wrist, hemorrhage, micromelia, distended chest, open eye, resorbed uterus, compressed, anophthalmia, exencephaly, macroglossia and omphalocoel has been observed, shown in Fig. 7.

3.2. Morphometric analysis

Means of fetal body weights, crown rump (CR) length, fore-limbs length, hind-limbs length and tail were calculated in the dose groups. Analysis of variance(Tukey/HSD (Honestly significant difference) had shown the analysis between all dose groups. Highest dose group (200 mg/kg BW) showed more trend of difference as compared to middle (133.3 mg/kg BW) and lowest (100 mg/kg BW) dose groups (Table 1).

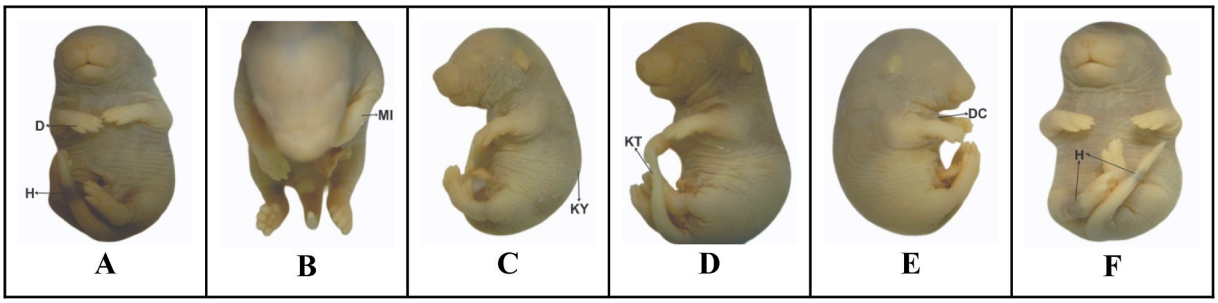

Fig. 5. Macro-photograph of 18 days old fetuses recovered from untreated dams.
Labels: D: Drooped, Hm: Hemorrhage, MI: Micromelia, KY: Kyphosis, KT: Kinked tail, DC: distended chest.

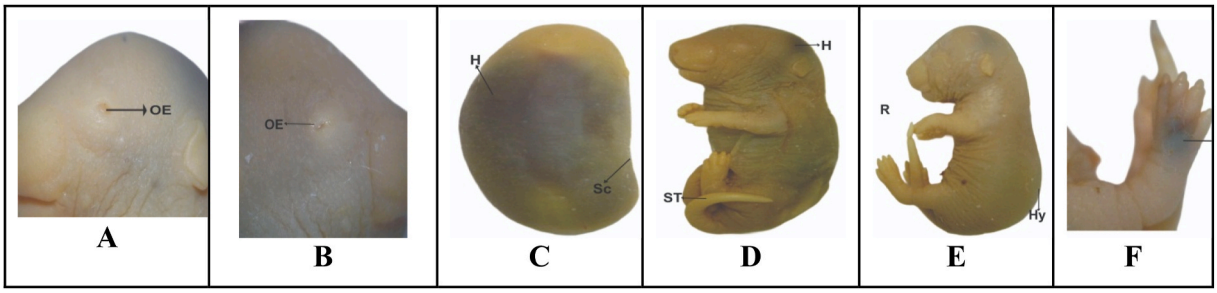

Fig. 6. Macro-photograph of 18 days old fetuses recovered from untreated dams.
Labels: Hm: Hemorrhage, OE: Open eye, Sc: Scoliosis, ST: Short tail, Hy: Hygroma, R: Runt.

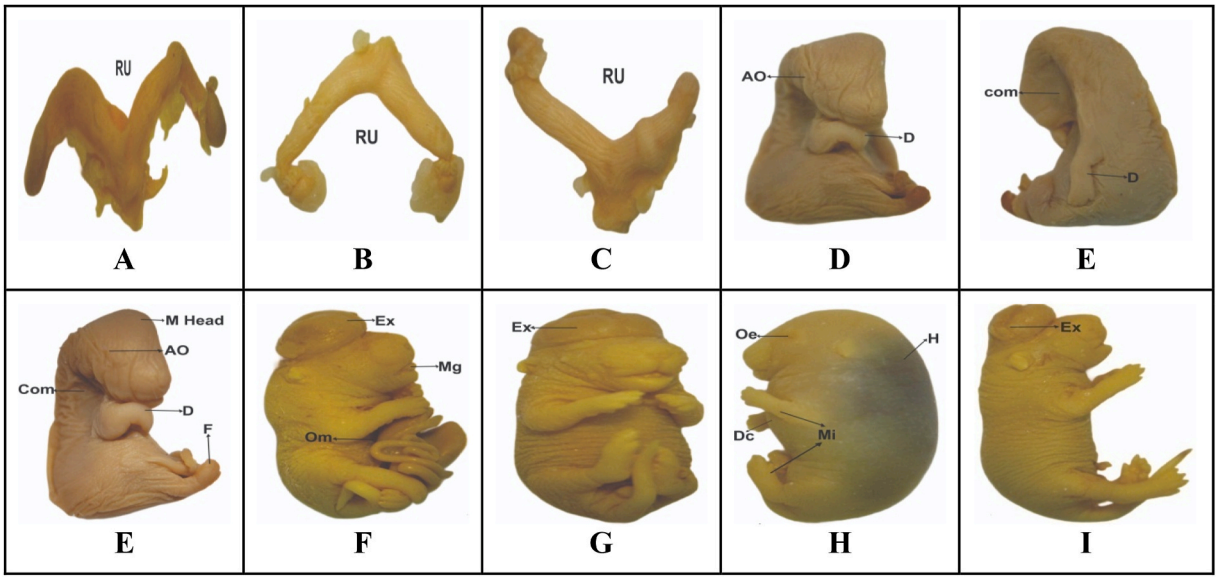

Fig. 7. Macro-photograph of 18 days old fetuses recovered from untreated dams.
Labels:D: Drooped, Hm: Hemorrhage, MI: Micromelia, DC: Distended chest, OE: Open eye, RU: Resorbed uterus, Com: Compressed, AO: Anophthalmia, Ex: Exencephaly, Mg: Macroglossia, Om: Omphalocoel.

3.3. Histological analysis

Fetal internal morphological studies of all dose groups (Control, 200 mg/kg, 133.3 mg/kg and 100 mg/kg) was done to find out anomalies caused by CuO-NPs in 18 days old fetuses. Selective sections are used here for group wise explanation of internal anomalies of fetuses.

The control groups show normal histological parameters as shown in Fig. 8. While low dose group (100 mg/kg BW) had fetuses with internal defects like anomalies in third ventricle, defected lateral ventricle, abnormal development of mandibular gland, misshapen nervous system and anomalies in nasal epithelium, bicuspid valve and distended Chest has been observed as shown in Fig. 9.

Medium dose group exposed to CuO-NPs shows abnormalities like microglossia, misshapen midbrain and lateral ventricle, nasal epithelium have disrupted cells, irregularities in cerebellum, lateral ventricles, olfactory bulb, eye and in tongue as shown in Fig. 10.

Likewise high dose of CuO-NPs show the following histological defects like misshapen third ventricle and lateral ventricle, irregularities in mandibular gland, and nasal epithelium nonstandard bicuspid valve, distended Chest, irregular spinal cord, damaged lungs and atria,

Table 1
Body weights in milligrams, CR length, forelimbs length, hindlimbs length and tail length observations of 18-days old fetuses recovered from pregnant mice administered with different concentrations. Those not sharing a common alphabet within a respective column are significantly different from each other

	Body weight (mg)	CR length (mm)	forelimbs length (mm)	hind limbs length (mm)	Tail (mm)
Control	1509.98 ± 49.06	22.37 ± 0.70	7.29 ± 0.85	9.59 ± 0.78	11.14 ± 0.50
D1 (200 mg/kg BW)	905.57 ± 117.61^{a,b,c}	18.23 ± 1.29^{a,b,c}	6.43 ± 0.29^{a,b}	8.25 ± 0.37^{a,b}	8.88 ± 0.61^{a,b,c}
D2 (133.3 mg/kg BW)	1139.90 ± 91.70^{a}	19.93 ± 1.29^{a,b}	6.55 ± 0.17^{a,b}	8.41 ± 0.42^{a,b}	10.15 ± 0.18
D3 (100 mg/kg BW)	1196.71 ± 196.56^{a}	21.11 ± 0.88	7.03 ± 0.08	9.12 ± 0.50	10.20 ± 0.44

anomalous liver and thymus, distorted urinary bladder and intestine, microglossia, molar abnormalities, abnormal development of humerus, aortic arc, fibula, tibia, stomach, and deposition of brown fat in the nervous system has been recorded as shown in Fig. 11.

3.4. Fetal skeletal examinations

Fetal skeletal studies of all groups (Control, 200 mg/kg, 133.3 mg/kg and 100 mg/kg) weremade to assess abnormalities caused by CuO-NPs in old fetuses of 18 days. To explain group-wise ossification anomalies of fetuses,macro-photographs are used here selectively.Well ossified with clear features and well deep stained structures were seen in all fetuses from the control group (Fig. 12). Yet dams treated with different dose concentrations show less or no ossification as shown in Figs. 13–15.

4. Discussion

The use of nanoparticles develops a general concern on their toxicity, when human and other animals are exposed directly or indirectly. The gravid females are especially vulnerable to NPs toxicity, moreover this exposure affects developing fetuses life. The CuO-NPs are considered highly toxic to living systems. It should be properly evaluated that how much it is dangerous or harmful for life before its use in various applications. Present study gives a deep insight into developmental toxicity in mice caused by CuO-NPs.The toxicity of CuO-NPs in animals is associated with their physical properties like size, solubility, surface area and its reactivity (Zhao et al., 2007). So size reduction results in increased surface area that not only abandons the particles in solution but also

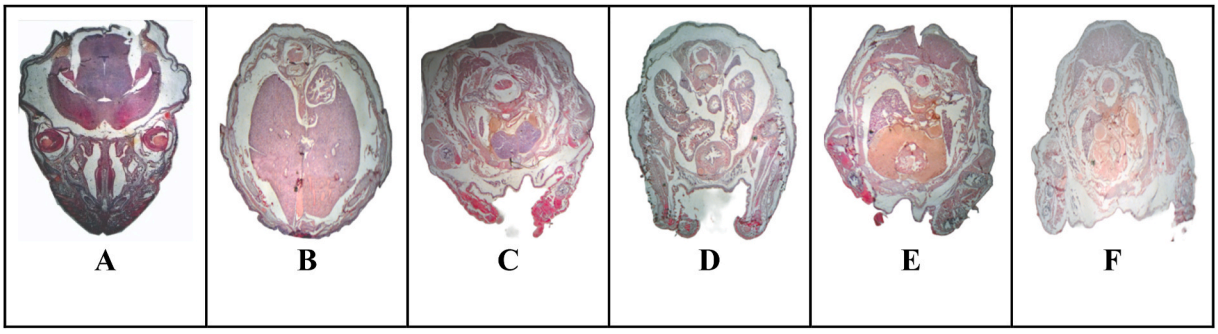

Fig. 8. Macro-photograph of 18 days old fetuses recovered from untreated dams.

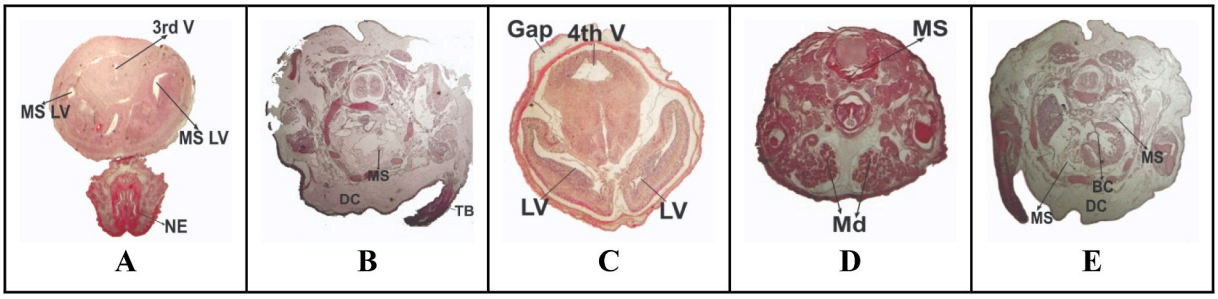

Fig. 9. Macro-photograph of 18 days old fetuses recovered from untreated dams.
Labels: 3rd V: third ventricle, LV: Lateral ventricle, Md: Mandibular gland, MS: Misshapen, NE: Nasal epithelium, BC: Bicuspid valve, DC: Distended Chest.

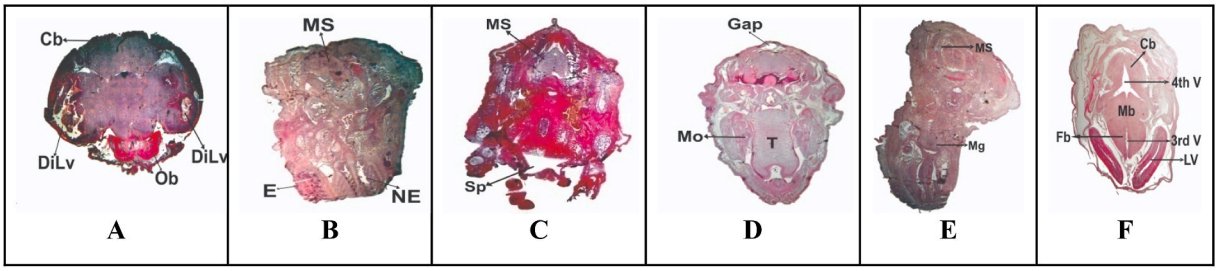

Fig. 10. Macro-photograph of 18 days old fetuses recovered from untreated dams.

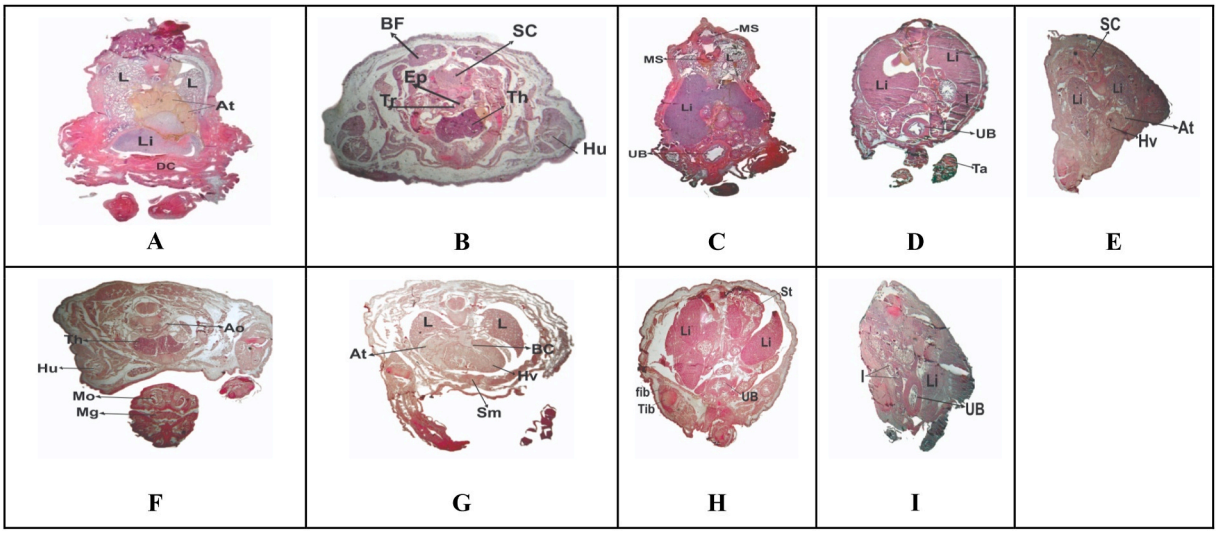

Fig. 11. Macro-photograph of 18 days old fetuses recovered from untreated dams.
Labels: 3rd V: third ventricle, LV: Lateral ventricle, Md: Mandibular gland, MS: Misshapen, NE: Nasal epithelium, BC: Bicuspid valve, DC: Distended Chest, SC: spinal cord, L; lung, At: Atria, Li: Liver, Th: Thyms, UB: Urinary bladder, I: Intestine, Hv: Heart ventricle, Mg: Microglossia, Mo: Molar, Hu: Humerus, Ao: Aortic arc, Fib: Fibula, Tib: Tibia, St: Stomach, BF: Brown fat. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

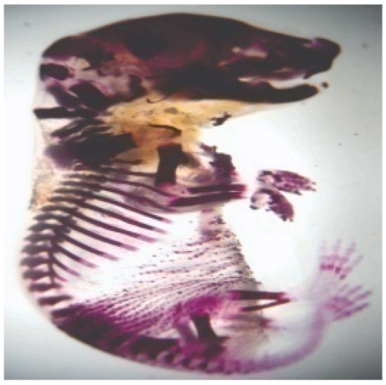

Fig. 12. A photograph of an 18 days old fetus recovered from the Control group showing well ossification throughout the skeleton.

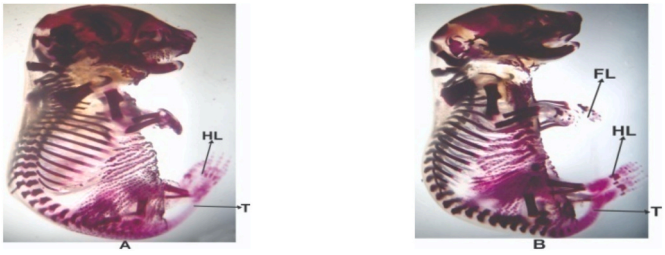

Fig. 13. Photographs "A" and "B"of 18 days old fetuses recovered from dams treated with 100 mg/kg BW (D1) showing less ossification in hind-limbs, Tail and fore-limbs.
Labels: HL: hind-limbs, T: Tail, FL: fore-limbs.

makes it possible to interact with molecules and increases the reactivity (Seyedalipour et al., 2015a, 2015b). Size, dosage and structure of nanoparticles greatly influence the extent of toxicity and their clearance from the body and reflected even at cellular level (Kadammattilet al., 2018).Our data has revealed that CuO-NPs greatly impair fetal development in many ways like morphological, histological etc. Recent studies on embryotoxicity and teratology including reproductive toxicity, neurotoxicity, immunotoxicity, renal toxicity, pulmonary injury, and hepatotoxicity have been reported (Wu et al., 2013; Lin et al., 2008; Chou et al., 2008; Wang et al., 2015a; Schipper et al., 2008; Bartneck et al., 2012).Present study emphasized its embryo-toxicity and teratological effects on mice. In this research it has been observed that it not only causes morphological but also morphometrical, histological and skeletal deformities, and is highly toxic for mice development. Its high concentrations are highly embryo-toxic and teratogenic as compared to low concentrations(Michaluk and Kochman, 2007; Ferm and Hanlon, 1974; Whittinghamet al., 1972). Researchers reported abnormal mice development in agreement with our present findings. High concentrations of CuO-NPs are demonstrated to induce fetal anomalies and resorption upon intravenous injection (Kadammattilet al., 2018). CuO-NPs exposure also resulted in hematoma, opened eyes and bent tail in the developing embryos (Liao and Liu, 2012). These above-mentioned studies are in collation to our findings. Roychoudhury et al. (2016) and Ferm and Hanlon (1974) studied the morphological effects of CuO-NPs and observed exencephaly, hemorrhages, hyperextension of limbs, Kinked and small tail which are according to our recent work. Our results have further been supported by the work of Roychoudhury et al. (2016) and Ganesanet al. (2016) who reported similar findings. Sharmaet al. (2020) and Rajendranet al. (2020), Abudayyaket al. (2020) reported that mice treated with CuO-NPs show edema, Hyperplasia, altered anatomy of intestinal villi and necrosis, even in the lowest dose group indicates cellular damage further support our results. Whole section through kidney show change in morphology and altered diameter of both proximal and distal tubules similar findings has been reported by Bugata et al. (2019) and Abou El-Nour et al. (2021).The malformation in 3rd ventricle, dilated lateral ventricle, cerebellum, olfactory bulb and deposition of brown fat in brain and change in spinal cord architecture is being observed in sections. Our findings are in coalition with previous studies carried out on mice development treated either orally or intraperitoneal injected CuO-NPs carried by Sajidet al. (2015) and Feliuet al. (2016). Win-Shwe andFujimaki (2011)reported accumulation of CuO-NPs in the brain via olfactory nerve route and cause anomalies to its various parts which are accorded to present study. CuO-NPs triggered necrosis and podocyte apoptosis of kidney tubules. Supporting these results, findings of Kadammattilet al. (2018) and Roychoudhuryet al. (2016) represent embryo-toxicity by CuO-NPs shortened body length and weight,

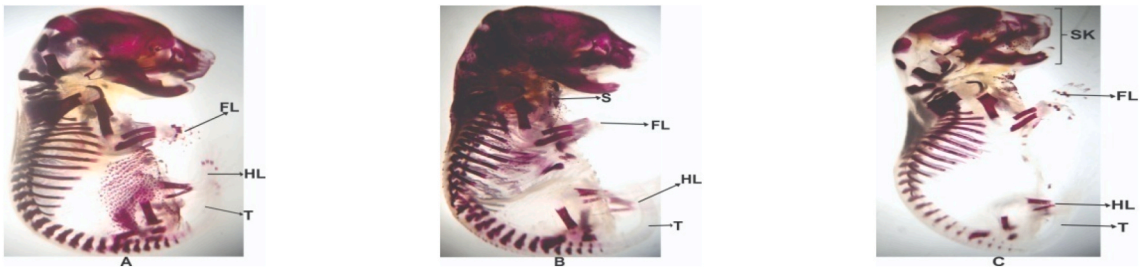

Fig. 14. (A, B, C): Photographs of 18 days old fetuses recovered from dams treated with 133.3 mg/kg BW (D2) showing less ossification in hind-limbs, Tail, fore-limbs, sternum and skull.
Labels: HL: hind limbs, T: Tail, FL: fore limbs, S: sternum, Sk: skull.

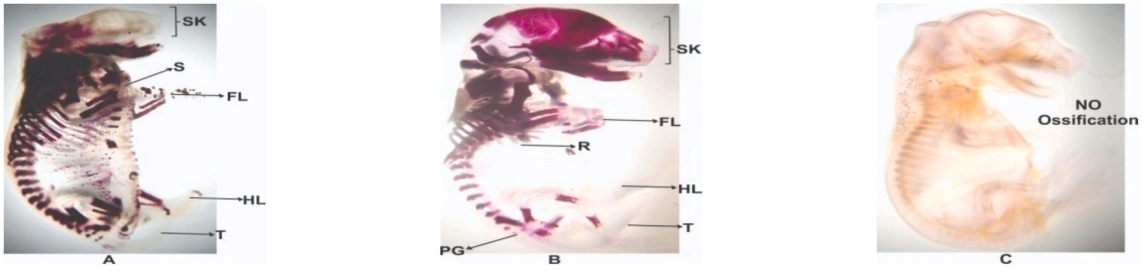

Fig. 15. "A" and "B" of 18 days old fetuses recovered from dams treated with 200 mg/kg BW (D3) showing less ossification in hind-limbs, Tail, fore-limbs, sternum, ribs and skull whereas "C" showing no ossification et al.
Labels: HL: hind limbs, T: Tail, FL: fore limbs, S: sternum, Sk: skull, PG: pelvic girdle, R: ribs.

hatching failure, hatching rate of embryos and lower reproduction rate. In other studies, carried out on murine, high toxicity of CuO-NPs has been observed in lung, kidney, liver, and in the nervous system. In conclusion CuO-NPs are very harmful. Histopathological changes of lungs, severe odema, severely cytotoxic inflammation and furthermore CuO-NPs induced intense deterioration of spleen and caused changes in kidney has been observed due to accumulation of CuO-NPs in the olfactory bulb (Cho et al. (2010), 2012b; Chen et al. (2006); Menget al., 2007; Pettiboneet al. 2008; Kimet al., 2011; Yokohiraet al., 2008).The uptake behavior of CuO-NPs in the cells and then simultaneously releasing relevant toxic ions in a bulk amount within cells (Trojan horse-type mechanism) accompany high toxicity level (Cronholmet al., 2013). The rich redox potential of CuO-NPs causes oxidative stress (OS) in cells. In CuO-NPs experienced cells, various marks of OS has been observed like stimulation of catalase, production of reactive oxygen species (ROS) and induction of superoxide dismutase (SOD) all leading in the lacerations in DNA (Karlssonet al., 2008; Ahamedet al., 2010). The releases of toxic Cu ions from CuO-NPs are one of the factors in the release of hydrogen peroxide (H_2O_2) locally at the plasma membrane resulting in the disruption of membranes (Karlssonet al., 2013; Vanwinkleet al., 2009). All above studies show that this NP has a toxic potential to cells and organisms.The group of pregnant rats inoculated intravenously with CuO-NPs displayed reduces body weight, forelimb growth and tail of embryos as compared to low dose group (Elkhateebet al., 2020). Seyedalipour et al. (2015a, 2015b) reported that CuO-NPs could cause morphometric anomalies in tail length, fore and hindlimbs as well in the reduction of crown rump length along with overall teratogenic effects.Another study carried by Zhang et al. (2017) showed that NPs significantly shortened pups body weight, and enhanced fetal resorption rate.

CRediT authorship contribution statement

Munir Ahmad: Investigation, Methodology, Writing – original draft. **Muhammad Khalil Ahmad Khan:** Conceptualization, Resources, Supervision. **Naveed Ahmad:** Software, Supervision. **Munazza Parveen:** Formal analysis. **Khurram Shahzad:** Validation. **Ali Hasan:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2023.114369.

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