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Original article

Maternal and developmental toxicity induced by Nanoalumina administration in albino rats and the potential preventive role of the pumpkin seed oil

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ABSTRACT

Although Nanoalumina is widely used in many biomedical applications, its potential toxic effect on pregnant women and developing embryos/fetuses has not been reported. In this investigation, the maternal and developmental toxicity caused by Nanoalumina during gestation and the potential preventive role of the pumpkin seed oil (PSO) were evaluated. Four groups of pregnant rats were orally administered during days 5–19 of gestation as follows: control group, Nanoalumina group (70 mg/kg b.w), PSO- group (4 ml/kg b.w.), and Nanoalumina plus PSO- group. Nanoalumina induced detrimental impacts in pregnancy outcomes, fetal growth retardation, morphological anomalies, hepatic and neural DNA damage, and histopathological changes in hepatic and neural tissues of both mother and fetus, respectively. Furthermore, the level of MDA is significantly increased and activities of GSH and CAT are significantly reduced in both tissues of nanoalumina-administered rats. PSO co administration improved pregnancy outcomes, fetal growth parameters, DNA damage, antioxidant defenses the histopathological changes of nanoalumina-gavaged rats and significantly diminished MDA level. Finally, PSO has a preventive role against the detrimental impacts of nanoalumina in dams and fetuses probably via its potential to prevent reactive oxygen species.

1. Introduction

Alumina Nanoparticles (Al_2O_3-NPs) are excessively used in industries of pharmacy as a carrier system to increase solubility of drug (Tyner et al., 2004), water treatment, and manufacturing such as manufacture of electrical components and batteries (Piercey and Klapoetke, 2010). Increasing concern of the bio safety with respect to public health, AlNP can permeate biological barriers and accumulate in many organs as shown in several studies (Almeida et al., 2011, Morsy et al., 2016b), causing neurotoxicity both *in vivo* and *in vitro* (Dong et al., 2011; Chen et al., 2013; Morsy et al., 2016a), genotoxicity (Balasubramanyam et al., 2009), in addition liver and kidney toxicity (Morsy et al., 2016a). Keelan (2011); Muoth et al., (2016); Hamdi (2020a,b) reported that NPs can penetrate the biological barriers, such as the blood–brain barrier (BBB), blood–testis barrier, and placental barrier. Zhang et al. (2018) showed that Alumina nanoparticles caused neurodevelopmental toxicity in offspring of exposed female mice during pregnancy, and they indicated that the contents of aluminum were localized in the newborns hippocampus. Nevertheless, no studies have denominated whether Al_2O_3-NPs induce maternal and developmental toxicity in rats during gestation.

Pumpkin seed oil (PSO) is a hygienic addition in diet of human and has potential appropriateness for both nutritional and industrial uses (Stevenson et al., 2007). Pumpkin seed oil ingredients are carotenoids, vitamin E and vitamin A as antioxidants, Omega-six, omega- nine fatty acid, and phytosterols (Murkovic et al., 1996). Linolenic acid, a polyunsaturated fatty acid is constituent of Pumpkin seed oil, which increases membrane fluidity, intracellular and extra cellular gaseous exchange and osmosis (Lovejoy, 2002). Also, Pumpkin seed oil contains fatty acids: linoleic (C 18:2), oleic (C 18:1), palmitic (C 16:0) and stearic (C 18:0) (Kulaitiene et al., 2007). Pumpkin has many health benefits such as antioxidant and anti-inflammation inflammation (Chen and Huang, 2018; Nawirska-Olszańska et al., 2013), cytoprotective

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(Shayesteh et al., 2017), and anti-mutagenic actions (Elfiky et al., 2012).

The current work was executed to estimate the possible maternal and developmental toxicity caused by Nanoalumina administration during gestation period and the teratogenic effect of it and the potential protective role of pumpkin seed oil against the toxicity caused by Nanoalumina administration.

2. Materials and methods

2.1. Experimental animals

Healthy mature female and male Wistar albino rats (***Rattus norvegicus***) (7–9 weeks old, 180 g----200 g b.w) were obtained from the animal house of the faculty of veterinary, Cairo university, Egypt. This study was approved by the Institutional Animal Care and Use Committee (CU-IACUC) of Cairo University (No. CU/I/F/61/19).

2.2. Materials

Nanoalumina: Al_2O_3-NPs was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA product number 544833, CAS number 1344–28-1). Nanoalumina was used in the ultrasonicated form, with diameter < 50 nm.

Pumpkin seed oil (PSO): 100% Natural Cold Pressed Pumpkin Seed Oil, obtained from Imtenan health shop, Egypt.

2.3. Characterization of aluminum oxide nanoparticles

2.3.1. Transmission electron microscopic (TEM) analysis

TEM [(TEM) Tecnai G2-200KV with microanalysis] to determinate of the size particle and shape of aluminum oxide nanoparticles. The sample preparation for TEM observation was as follow: Al_2O_3-NPs powder was firstly dissolved in dist water, the aqueous suspension was ultrasonicated and the ultrasonic dispersed particles were deposited on the lacey-carbon-coated copper grid.

2.3.2. Powdered X-ray diffraction analysis

The aluminum oxide nanoparticles crystal structure was estimated by powder XRD (D8 Advanced X-ray Diffractometer, Burker, Germany). About 250 mg of Al_2O_3 was deposited on the sample holder for scanning over a range of 10–100 °C. Scanning was performed with 2.2 kW Cu anode radiations at a wavelength of 1.54 A° and at kV and 30 mA (Piercey and Klapoetke, 2010).

2.4. Experiment design

After the acclimatization period of rats for 7 days, two females were coexisted with a male overnight in suitable cages; the presence of sperm in the vaginal smears indicate the successful mating and the day zero of pregnancy. Twenty four pregnant rats were haphazardly assigned into four equal groups (6 rat /group), pregnant rats were received doses once daily via gastric tube from 5th till 19th day of gestation. Group 1: Control group, pregnant animals were received 1 ml of distilled water. Group 2: Pumpkin Seed Oil group, pregnant animals were received 4 ml/kg oil with reference to Sayed (2014). Group 3: *Nanoalumina* group, Al_2O_3-NPs was dissolved in dist water (aqueous suspension). Pregnant animals were received 1 ml dist water containing 70 mg/kg bw (Ultrasonicated aqueous suspension) with reference to Yousef et al. (2019). Group 4: co administered group, pregnant animals were received Pumpkin Seed Oil plus aqueous suspension of Al_2O_3-NPs in similar doses as in group two and three. The sacrificed pregnant rats by decapitation were exposed to cesarean section on day 19 of gestation; the two uterine horns were removed and weighted. Total implantation sites, Corpora lutea number, live and dead fetuses and the pre/post implantation loss were recorded. The placentas were examined and their weights were recorded. Live fetuses were removed from the uterus, and fetal body weight, body length, tail length were recorded, and examined for gross malformations El Ghareeb et al. (2015).

2.5. Comet assay

DNA damage was investigated in maternal and fetal tissues (liver and brain) of all experimental groups using the alkaline comet assay according to the method described by (Tice et al., 2000).

2.6. Oxidative stress assay

Supernatants of hepatic and neural homogenates of both mother and fetus of all experimental groups were used to the estimation of malondialdehyde (MDA) level, glutathione (GSH) concentration and catalase (CAT) activity according to methods described by (Ohkawa et al., 1979), (Beutler et al., 1963) and (Aebi 1984) respectively.

2.7. Histopathological assay

Maternal and fetal tissues (liver and brain) of all experimental groups were fixed in 10% neutral formalin, processed and stained with haematoxylin and eosin (Bancroft and Gamble, 2008) for histological examination under light microscope.

2.8. Statistical analysis

The present data were analyzed for statistical significance by the one-way analysis of variance, followed by Tukey's multiple comparison tests. Statistical analysis of data was performed using GraphPad Prism 5. Data were expressed as mean ± standard error (*SE*). The data at $p < .05$ were considered significant.

3. Results

3.1. Al_2O_3-NPs characterization

(Fig. 1) represented the characterization of Al_2O_3 –NPs done by TEM and X-ray diffraction analysis. Al_2O_3–NPs had very thin particles (nanopowder, < 50 nm) obtained by TEM measurements. Five dominant peaks [32.9°, 36.65°, 39.312°, 45.54° and 67.307°] seen by XRD results. These data obtained were matched with the database obtained by Pakrashi et al., (2013), which affirm the crystalline structure of alumina.

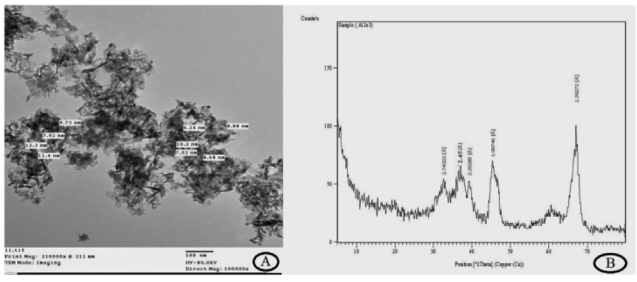

Fig. 1. Characterization of Aluminium Oxide Nanoparticles by TEM (A) and X-ray diffraction pattern (B).

Table 1
Influence of Pumpkin seed oil on Pregnancy outcome and growth parameters in pregnant rat administrated pumpkin seed oil and/or Al_2O_3-NPs at the 19th day of gestation. a: Statistically changed from the control rats; b: Statistically changed from the Al_2O_3-NPs administrated rats.

	Groups			
Parameters	Control	PSO	Al_2O_3-NPs	PSO + Al_2O_3-NPs
No. pregnant rats	6	6	6	6
No. of implantation sites/ litter	8.5 ± 0.6455	8.25 ± 0.4787	6.5 ± 0.2887[a]	7.5 ± 0.2887
No. Live fetuses/ litter	8.5 ± 0.6455	8.25 ± 0.4787	4 ± 0.9129[a]	6.5 ± 0.2887
Postimplantaion loss index-%	0 ± 0	0 ± 0	39.88 ± 11.68[a]	12.88 ± 5.117[b]
Gravid uterus weight (g)	40.13 ± 0.696	40.98 ± 0.6045	23.26 ± 1.414[a]	34.24 ± 0.2272[b]
Mother weight gain (g)	59.33 ± 0.8819	60 ± 0.5774	39.67 ± 2.333 [a]	50.67 ± 0.3333[b]
Fetal length (cm)	5.214 ± 0.2551	5.312 ± 0.2681	3.692 ± 0.1292 [a]	4.6 ± 0.05773[b]
Tail length (cm)	1.418 ± 0.0777	1.432 ± 0.04189	1.079 ± 0.01667 [a]	1.367 ± 0.03333[b]
Fetal weight (g)	3.568 ± 0.1954	3.977 ± 0.04743	1.152 ± 0.1505 [a]	2.867 ± 0.06667[b]
Placenta weight (g)	0.5569 ± 0.05508	0.6059 ± 0.05301	0.1787 ± 0.06155 [a]	0.4 ± 0.01155[b]

3.2. Morphological investigation

3.2.1. The influence of PSO and/orAl_2O_3-NPs on dam during the gestation period

(Table 1) revealed that body weight gain, uterine weight, the average weight of placenta, implantation sites and the number of live fetuses were significantly decreased in the pregnant rats administrated with Al_2O_3-NPs alone compared with the control group. In addition, a marked increment in post implantation loss / litter in Al_2O_3-NPs administered rats compared with the control ($P > 0.05$). Meanwhile, co-administration of PSO plus Al_2O_3-NPs significantly mitigated the reduction in the body weight gain, uterine weight, the average weight of placenta, and significantly elevated the implantation sites and the live fetuses' number. Furthermore, pumpkin seed oil co administration significantly and insignificantly declined the percentage of post implantation loss/litter, respectively, throughout the administration period. Moreover, no significant difference was noticed in the above parameters in pregnant rats received PSO compared with the control.

Fig. 2 revealed that the two uterine horns of pregnant rats received PSO had normal distribution of fetuses identical to that of the control group. However, fetuses were partially distributed in the two uterine horns (only on one horn) and decreased number of fetuses implanted in the gravid uterus of the rats administered Al_2O_3-NPs alone compared to the control group. Meanwhile, the co-administration of PSO plus Al_2O_3-NPs could normalize distribution of fetuses in the two uterine horns.

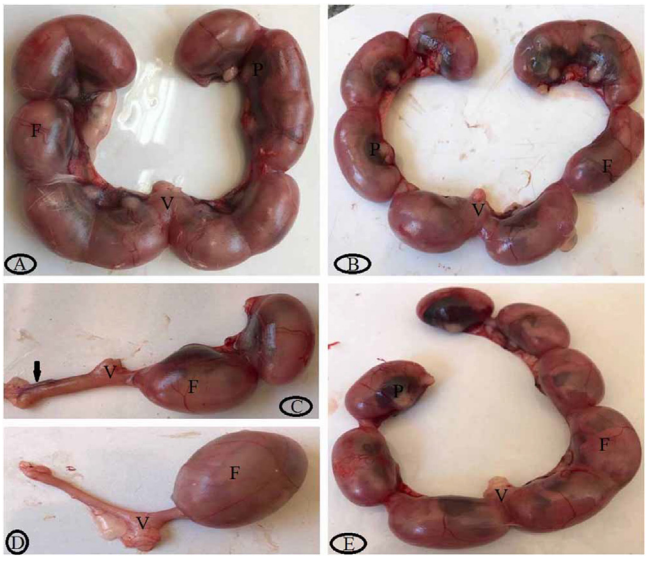

Fig. 2. Photographs of uterus of pregnant rat at the 19th day of gestation. control (A), PSO (B), Al_2O_3-NPs (C&D) and PSO + Al_2O_3-NPs (E). Fetuses are normally distributed in the two uterine horns (A&B). Pinpoint hemorrhagic implantation sites (early resorption) (arrow) are observed in one uterine horn (C). Only one fetus is observed in one uterine horn (D). Asymmetrical distribution of fetuses more or less normal (E). F = fetus, V = vagina, P = placenta.

3.2.2. The influence of PSO and/orAl_2O_3-NPs on fetal growth parameters

Fetal body length, weight, and tail length in the group administrated Al_2O_3-NPs were decreased significantly compared to the control group. However, PSO alone showed insignificant difference compared with control. Co-administration of Al_2O_3-NPs with PSO could improve the fetal growth parameters compared to Al_2O_3-NPs alone– administrated group, although they were still lower than the control (Table 1).

3.3. Morphological anomalies

Control and PSO maternally administrated fetuses revealed normal size, length and morphological appearance (Fig. 3A, B). However, the most repeated anomalies observed in the Al_2O_3-NPs administrated group were the subcutaneous hematoma in different sites, kinky tail, absence of digits, microcephaly and short snout (Fig. 3C–F). The co– administrated group showed an evident decrease in the incidence of the malformations observed in Al_2O_3-NPs administrated group (Fig. 3G).

3.4. Comet assay

In the present work, toxicity of Al_2O_3-NPs was characterized by a significant increment in amount of DNA in the comet tail of hepatic and neural cells of both mother and fetus (Fig. 4). A significant ($p < .05$) increase in percentage of DNA, tail length and tail moment (parameters of comet assay) in maternal and fetal hepatic and neural cells of Al_2O_3-NPs administrated pregnant rats in respect to control (Table 2). Co administration of PSO resulted in significant decline in the parameters of comet of pregnant rats. PSO had a remarked preventive role against DNA damage induced by Al_2O_3-NPs.

3.5. The influence of PSO on Al_2O_3-NPs -induced oxidative stress in the hepatic and brain tissues

Al_2O_3-NPs administration caused a significant elevation in hepatic and neural MDA (lipid peroxidation by-product), a significant decline in hepatic and neural GSH, and CAT levels in both mother and fetus as compared with control rat. Meantime, significant improvements ($p < .05$) in the levels of the above mentioned markers of oxidative stress were recorded in the fetal, maternal hepatic and brain homogenates of PSO - co administrated pregnant rat (Fig. 5).

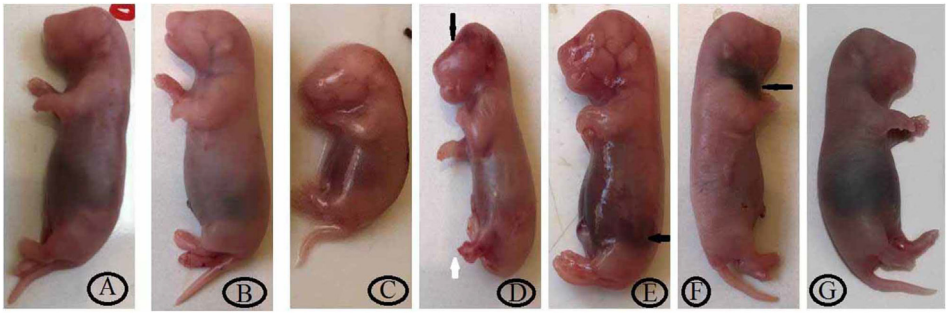

Fig. 3. Photographs of fetuses at 19th day of gestation. control (A), PSO (B), Al_2O_3-NPs (C-F) and PSO + Al_2O_3-NPs (G), normal fetus (A&B), fetus with diminution in size and kinky tail(C), fetus with head hematoma(black arrow) and absence of digits (white arrow)(D),abdomen hematoma(E), fetus with microcephaly, short snout and neck hematoma (arrow) (F), normal fetus (G).

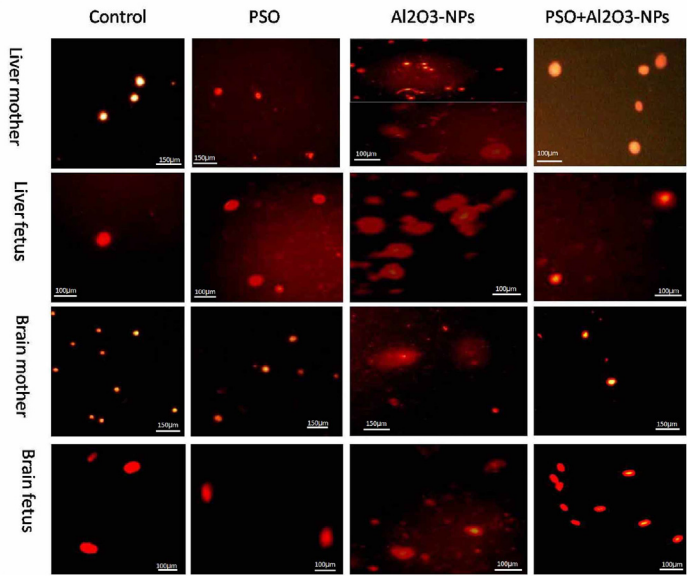

Fig. 4. Photomicrographs of comet assay showing, typical nuclei of undamaged hepatic and neural cells of control and PSO groups; DNA damage observed as comets in Al_2O_3-NPs and (PSO + Al_2O_3-NPs) groups.

Table 2
The percentage of DNA damage (%DNA), tail length (TL), and tail moment (TM), in the liver and brain of rat administrated pumpkin seed oil and/or Al_2O_3-NPs. a: Statistically changed from the control rats; b: Statistically changed from the Al_2O_3-NPs administrated rats.

Parameters / Organs		Experimental groups			
		Control	PSO	Al_2O_3-NPs	PSO + Al_2O_3-NPs
DNA%Tail	Maternal liver	2.643 ± 0.92	2.559 ± 0.06846	8.066 ± 0.3405[a]	5.515 ± 0.2496[b]
	Fetal liver	4.084 ± 0.2197	3.718 ± 0.2197	6.726 ± 0.1798[a]	4.881 ± 0.1277[b]
	Maternal brain	2.714 ± 0.3804	2.683 ± 0.6697	7.413 ± 0.1758[a]	5.097 ± 0.07894[b]
	Fetal brain	3.973 ± 0.08518	3.734 ± 0.002551	7.5 ± 0.1079[a]	5.248 ± 0.2047[b]
Tail Length (μm)	Maternal liver	3.467 ± 0.3531	3.198 ± 0.1103	13.86 ± 1.363[a]	7.373 ± 0.6371[b]
	Fetal liver	4.574 ± 0.3056	4.327 ± 0.09457	8.867 ± 0.2728[a]	5.743 ± 0.3055[b]
	Maternal brain	4.167 ± 0.09613	4.09 ± 0.06406	10.3 ± 1.212[a]	6.916 ± 0.3471[b]
	Fetal brain	3.967 ± 0.06009	3.676 ± 0.2728	8.24 ± 0.5108[a]	5.743 ± 0.3055[b]
Tail moment (μm)	Maternal liver	0.1308 ± 0.04833	0.1287 ± 0.01111	0.8792 ± 0.09704[a]	0.4178 ± 0.0726[b]
	Fetal liver	0.2515 ± 0.02326	0.241 ± 0.02088	0.7371 ± 0.03364[a]	0.3852 ± 0.01675[b]
	Maternal brain	0.1343 ± 0.005015	0.1252 ± 0.005106	0.9309 ± 0.08811[a]	0.3061 ± 0.03017[b]
	Fetal brain	0.2442 ± 0.01324	0.2352 ± 0.009762	0.524 ± 0.02952[a]	0.3719 ± 0.01161[b]

3.6. Histopathological results

3.6.1. Liver of pregnant rats

Microscopic examination of control liver of pregnant rats revealed the normal architecture (Fig. 6A). The liver of the pregnant rats administered PSO exhibited normal histological structure similar to that of the control group (Fig. 6B). The hepatic tissue of Al_2O_3-NPs administrated rats revealed marked tissue alterations, fatty degeneration, focal hepatic necrosis associated with lymphatic infiltration and vacuolization of hepatocytes cytoplasm (Fig. 6C, D). In co administrated rats, improvement in histopathological investigation was shown (Fig. 6E).

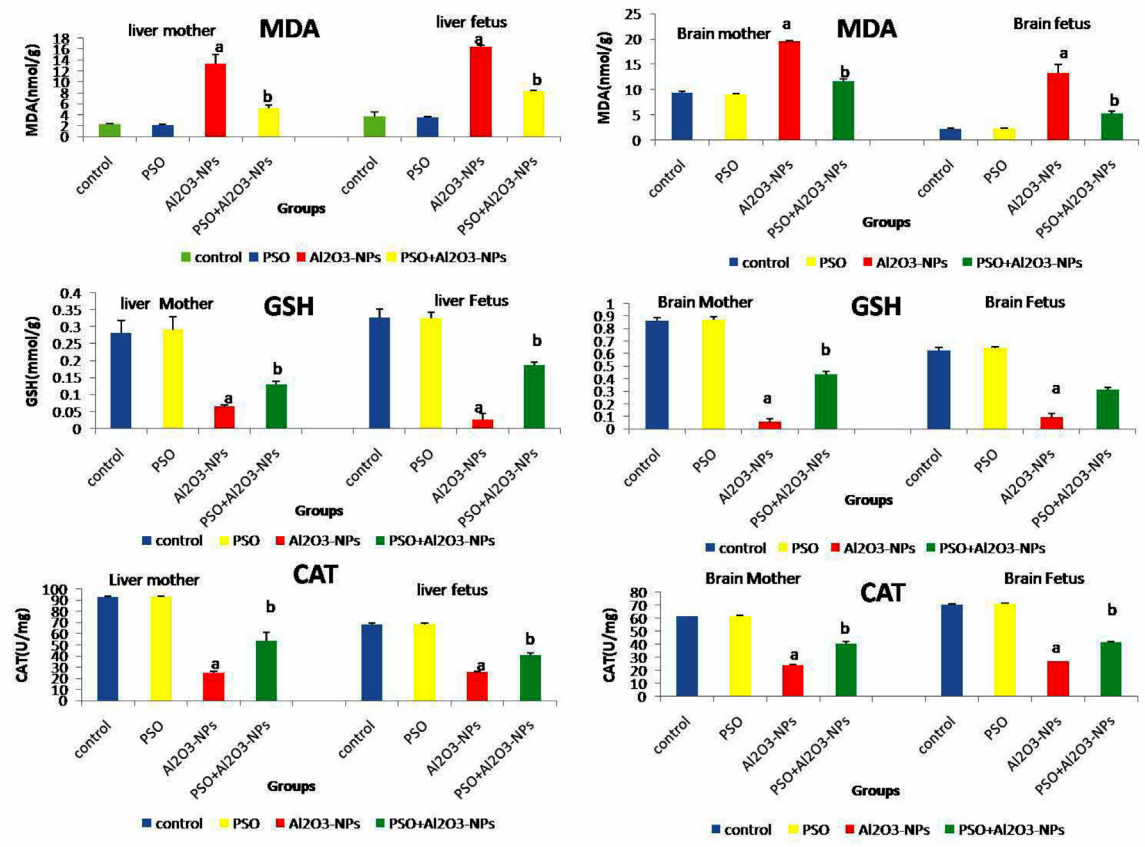

Fig. 5. Influence of Pumpkin seed oil on MDA, GSH and CAT levels in both maternal and fetal hepatic and brain tissues of pregnant rat administrated Al_2O_3-NPs. a: Statistically changed from the control rats; b: Statistically changed from the Al_2O_3-NPs administrated rats.

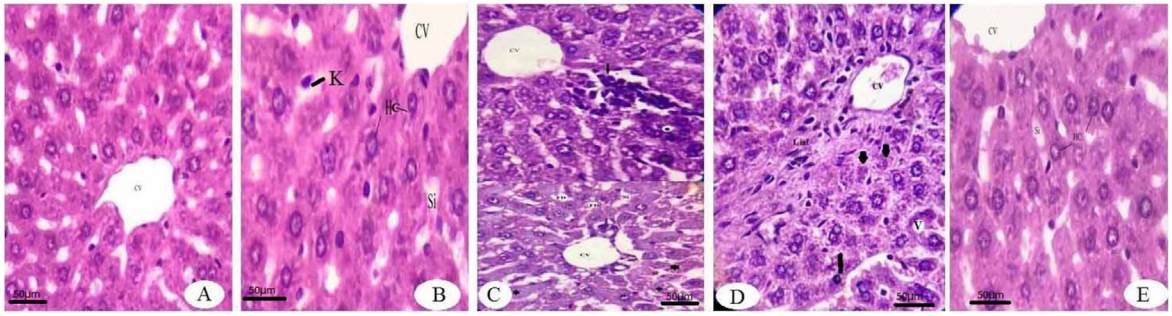

Fig. 6. Photomicrographs of liver sections of pregnant rats. control (A), PSO (B), Al_2O_3-NPs (C&D) and PSO + Al_2O_3-NPs (E). Normal architecture of maternal liver (A&B), focal hepatic necrosis associated with lymphatic infiltration (Linf) (arrow).Congested central vein (CV) Karyorrhextic (short arrow) ,Pyknotic hepatocyte (asterisk), hepatocytes (HC) exhibit fatty degeneration (FD)(C&D), almost normal architecture of maternal liver(E).blood sinusoids (Si).

3.6.2. Liver of fetuses

Fetal liver of control and PSO administered mothers revealed normal histological features (Fig. 7A, B). Fetuses maternally administered with Al_2O_3-NPs induced hepatocytes necrotic changes in the form small dense pyknotic nuclei characterized by condensed chromatin and pale vacuolated cytoplasm and numerous vacuoles including fatty degeneration. In addition, widened, and congested central vein with detached epithelium (Fig. 7C, D). Fetal liver of PSO co administrated mothers displayed a hepatic architecture more or less similar to the control group (Fig. 7E).

3.6.3. Brain of pregnant rats

Examination of the cerebral cortex sections of the control and PSO groups revealed normal architecture (Fig. 8A, B). However, the pyramidal cells with deeply stained nuclei, irregular in shape, their processes were lost and perineural spaces surrounded pyramidal and granule cells were observed in the cerebral cortex of Al_2O_3-NPs administrated rats. In addition, pyknotic neurons, vacuolization of the neuropil, dilated and congested blood vessels were seen (Fig. 8 C). In co administered rats, improvement in histopathological investigation was observed (Fig. 8D). The cerebellar cortex sections of the control and PSO groups revealed normal architecture (Fig. 9A, B). However, perineural spaces surrounded stellate cells and vacuolated areas were seen in the molecular layer in the cerebellar cortex of Al_2O_3-NPs administrated rats, the nuclei of Purkinje cells appeared dark stained with eosinophilic cytoplasm. In addition, a lot of Purkinje cells are lost leaving empty spaces. Granular layer appeared thin, dilated and congested

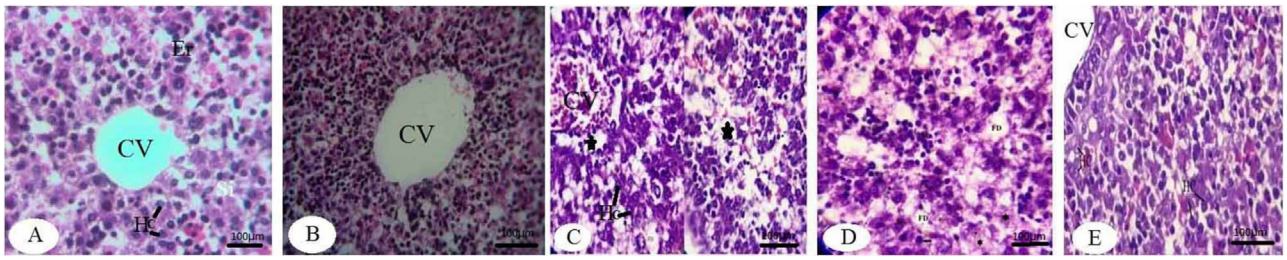

Fig. 7. Photomicrographs of liver sections of fetuses. Control (A), PSO (B), Al_2O_3-NPs (C) and PSO + Al_2O_3-NPs (D). Normal architecture of fetal liver (A&B), Congested central vein (CV) with detached epithelium. Pyknotic (arrow), Necrotic area in between the hepatic tissue (asterisk), hepatocytes (HC) exhibit severe fatty degeneration (FD)(C&D), almost normal architecture of fetal liver(E).

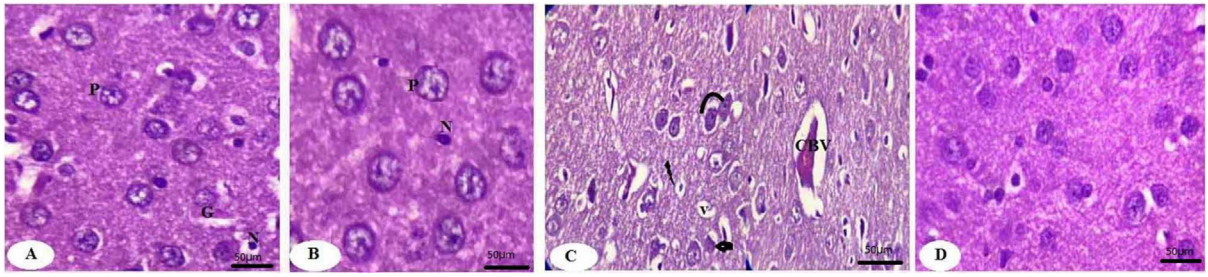

Fig. 8. Photomicrographs of the cerebral cortex sections of pregnant rats. control (A), PSO (B), Al_2O_3-NPs (C) and PSO + Al_2O_3-NPs (D).More or less normal pyramidal cells (P) , granule cells(G) and neuroglial cell(N)(A&B). Degenerated areas showed perineural spaces (wavy arrow), dark stained nuclei (curved arrow), pyknotic neuron (arrow),vaculatization of neuropil(V)and dilated congested blood vessels(CBV)(C). Relatively normal architecture (D).

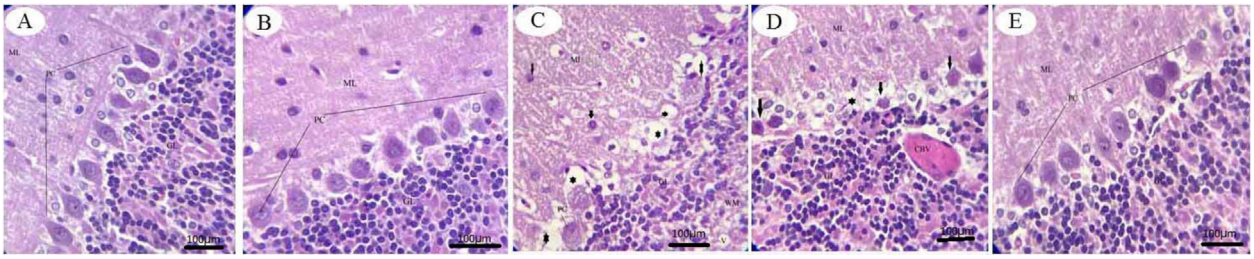

Fig. 9. Photomicrographs of the cerebellar cortex sections of pregnant rats. Control (A), PSO (B), Al_2O_3-NPs (C&D) and PSO + Al_2O_3-NPs (E). The layers of the cerebellum: The molecular layer (ML), Purkinje layer having large flask-shaped Purkinje cells (PC) and granular layer (GL). Normal architecture (A&B), a lot of Purkinje cells (PC) are lost leaving empty spaces (asterisk), stellate cells being surrounded by perineural spaces and vacuolated areas in molecular layer (asterisk)(C). Purkinje cells (arrows) with an eosinophilic cytoplasm, congested blood vessels (CBV) in granular layer(D) . Relatively normal architecture (E).

blood vessels were seen (Fig. 9C, D). In co administrated rats, an improvement in the histological structure was seen (Fig. 9E).

3.6.4. Brain of fetuses

Fetal cerebral cortex of control and PSO administered mothers revealed normal architecture, The cerebral cortex of the developing rats consisted of five basic zones. These zones, from outwards inwards were marginal (MZ), cortical plate (CP), intermediate (IZ), subventricular (SVZ) and ventricular (VZ) (Fig. 10A, B). Cell necrosis, vacuolization of the neuropil, dilated and congested blood vessels were seen in the cerebral cortex different zones of fetuses maternally administered Al_2O_3-NPs (Fig. 10C). Fetuses of PSO co administrated mothers revealed an improvement in the histological structure of cerebral cortex (Fig. 10D).

4. Discussion

The current work showed that Al_2O_3-NPs caused a significant decline in weight gain of mothers, their uteri, number of live fetuses and implantation sites, a significant increment in post implantation loss / litter and a significant decline in the growth parameters of fetuses, these data are in conformity with the findings of many previous studies noted the reproductive/developmental toxicity of other nanoparticles. Yamashita et al., (2011) reported that pregnancy complications as the smaller size of both uteri and fetuses caused by silica and titanium dioxide NPs administrated intravenously to the pregnant mice. Durnev et al. (2010) Showed that the injection of 50 mg/kg/day of silicon crystal, caused a decline in the body weight gain of pregnant rats and newborn rats at the different experimental stages, but they had no impact on the other parameters of physical development of rat progeny and caused no teratogenic effects. Otherwise, the oral administration of silver NPs (250 mg/kg/day) induced a comparatively low toxic impact Hong et al. (2014).

Hematoma, hind limb defects, short snout, microcephaly were the most repeated anomalies observed in the Al2O3NPs administrated group as seen in the previous study of El Ghareeb et al. (2015).

The current data revealed remarkable elevations of %DNA, TL, and TM in maternal and fetal hepatic and brain tissues of rats administered Al_2O_3-NPs. Jennifer and Maciej (2013) reported that Al_2O_3-NPs and ZnONPs caused genotoxicity and cytotoxicity in

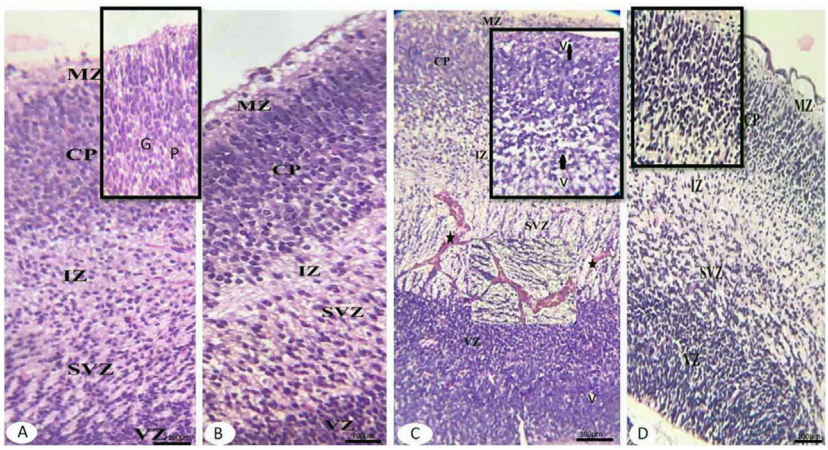

Fig. 10. Photomicrographs of the cerebral cortex sections of the brain of rat fetuses from control (A), PSO (B), Al_2O_3-NPs (C) and PSO + Al_2O_3-NPs (D). the cortical layers MZ, marginal zone; CP, cortical plate; IZ, intermediate zone; SVZ, subventricular zone; VZ, ventricular zone.(C): different nerve cells in the cerebral cortex. G: Granule cells, P: pyramidal cells , karyolytic nucleus (long arrow), pyknotic nuclei of nerve cells(small arrow),Vacuoles (V), dilated and congested blood vessels(asterisk). (D): almost normal nerve cells and neuropil.

hepatic cells. Al_2O_3-NPs can cause DNA damage indirectly through inflammation (Chen et al., 2006) and the generation of reactive oxygen species (ROS) (Federici et al., 2007, Gurr et al., 2005, Morsy et al., 2016c). The reactive oxygen radical is highly capable of oxidising the single base and sugar phosphate of DNA and breaks its strand (Bjelland and Seeberg, 2003, Cadet et al., 2003) . Marked elevations of %DNA, TL, and TM assured that the Al accumulated by the liver and brain cells was probably the main route by which nano-sized Al causes cytotoxicity.

In the present work, toxicity of Al_2O_3-NPs was characterized by histopathological changes. Furthermore, Al_2O_3-NPs administration caused a significant elevation in hepatic and neural MDA (lipid peroxidation by-product), a significant decline in hepatic and neural GSH, and CAT levels in both mother and fetus.

In accordance with the former studies, (Morsy et al., 2016a,b,c) Al_2O_3NPs administration caused a state of oxidative stress in liver and brain tissues of the rats, shown by increased lipid peroxidation and compromised antioxidant defense system. The changes in the biological membrane permeability and fluidity are attributed to the Lipid peroxidation which in turn can significantly affect cell integrity (Dix and Aikens, 1993).

It has been reported that Al_2O_3NPs induced tissue damage is mainly due to reactive oxygen species (ROS) production like hydrogen per-oxide, hydroxyl radical species, nitric oxide or superoxide anion (Li et al., 2008), Or may be due to its direct interaction with cell organelles, the formation of chemical compounds with DNA, RNA, proteins and so on, and by its accumulation in cells, tissues, and organs, leading to oxidative damage of organs (Morsy et al., 2016a).

Our findings suggested that NP passes the placenta, they may exert toxicity directly through production of ROS and inflammation in fetal tissue in conformity with (Lim et al., 2011), who reported that fetal malformations are related to the increased ROS in both the placenta and fetus.

Consistent with the current data, it has been suggested that nanoparticles enter a pregnant woman's body through inhalation, venous injection, and ingestion or skin permeation, maternal toxic stress reactions such as reactive oxygen species (ROS), inflammation, apoptosis and endocrine dyscrasia are caused in various organs, especially in the reproductive organs. the passive diffusion or endocytosis mechanisms by which some NPs can pass through the placenta into the fetus, and can induce inflammation, apoptosis, genotoxicity, cytotoxicity, low weight, reproductive deficiency, nervous damage, and immunodeficiency in fetus as reported by Hou and Zhu (2017).

The protective effect of pumpkin seed oil against maternal and development toxicity induced by aluminum oxide nanoparticles in pregnant rats could be owing to the biological benefits of its composition. It neutralizes and prevents the elevated ROS production and modulates DNA oxidative damage (Andjelkovic et al., 2010, Nyam et al., 2009). The antioxidant and hepatoprotective actions of pumpkin due to the powerful antioxidant polyphenol and B carotene content (Oboh 2005). PSO has anti-inflammatory action, attributed to its promising proportions of ω-6 and ω-9 UFAs which exerts its action either by their individual activity or the synergistic effect of these bioactive molecules (Saraiva et al., 2011). Furthermore, phenolic compounds (Andjelkovic et al., 2010) bind to free radicals and prevent the cell membrane oxidation and production of lipid peroxidation. Also, the phytosterols have antioxidant activity and they can reduce LDL-cholesterol which leads to the reduction of the lipid peroxidation (Nyam et al., 2009).

5. Conclusion

The present investigation concluded that Al_2O_3-NPs caused maternal and developmental toxicity by generating oxidative injury. Otherwise, pumpkin seed oil by elevating antioxidant activities and diminishing lipid peroxidation may provide protection against oxidative stress and may preserve the safety of tissue functions generated by Nanoalumina.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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