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Teratogenic Effects of the Titanium Dioxide Nanoparticles on the Pregnant Female Rats And Their Off Springs.

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ABSTRACT

Titanium dioxide TiO_2 nanoparticles are manufactured worldwide in large quantities for use in a wide range of applications including pigment and cosmetic manufactures. Although TiO_2 is chemically inert, nanoparticles can cause negative health effects. The present study investigates the teratogenic effects of TiO_2 on pregnant albino rats and their fetuses. Pregnant albino rats (*Rattus norvegicus)* were injected intraperitoneally with TiO_2, 0.5 mg/kg/day, from the5thday of gestation till the end of lactation. The animals were sacrificed at the end of gestation and during lactation. Fetuses were removed from the uterus and evaluated for mortality rate, growth parameters, morphological and skeletal malformations as well as histological study of brain, liver and kidney. The data revealed that fetal weights were significantly reduced in most study groups. It was found that severe degenerative changes were observed in the liver, kidney as well as the brain following TiO_2 administration.Titanium dioxide pretreatment was able to increase the level of lipid peroxidation significantly. The correlation noted between GSH levels and the Titanium dioxide effects is significant. Our findings suggest the need for great caution to handle the nanomaterials as Titanium dioxide especially during pregnancy and lactation.

Keywords: Nanoparticles, Titanium dioxide, Teratogenicity, Pregnant rats.

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INTRODUCTION

The Teratology is the study of abnormalities of physiological development (Rogers and Kavlock, 1996). Teratogenic agents cause approximately 7% of congenital malformations. A teratogenic agent is a chemical, infectious agent, physical condition, or deficiency that, on fetal exposure, can alter fetal morphology or subsequent function. Teratogenicity depends upon the ability of the agent to cross the placenta. Certain medications such as heparin cannot cross the placenta due to its high molecular weight and are therefore not teratogenic (Ji et *al*., 2010).

A chemical teratogen must be present in the mother's bloodstream at an effective exposure level at the wrong time. But only about 50 chemicals have been confirmed as human teratogens and only about 10% of birth defects are thought to be associated with environmental factors (Guidotti , 2010).

Nanoparticles (NP) are a class of organic or inorganic substances with the size range of 1-100 nm (Zhao and Castranova , 2011), they may form naturally, be produced as a waste product by human activity (automobile exhaust gases or emissions of power plants) or specifically engineered for industrial or medical purposes .

Nanotechnology is a rapidly developing and expanding field leading to an increase of engineered NP with conceptually new physical and chemical properties, which might induce effects in biological system (Hardman , 2006 and Gupta , 2007) .

Natural NPs have been present in the environment for million years ago in such forms as viruses and volcanic ash, and are sometimes known as "free" NPs, since they can exist in an unbound state. Manufactured, or engineered, NPs can be divided into different material classes, including metals, metal oxides, non-metals, polymer-based, carbon-based as well as those classified as semi-conductor materials, such as quantum dots (Klaine *et al*., 2008). Therefore comprehensive knowledge about possible physiological effect of NP is crucial independently on whether NP exposure is intended or not. Some nanoparticles such as titanium dioxide (TiO_2) or silica nanoparticles have been already used in cosmetics , food , electronics and medicine (Yamashita *et al* ., 2011).

The growing number of commercial products and expansion of NP application areas raise concerns about NP accumulation, long-term relation in an organism and subsequent toxic effect (Li *et al*., 2010).

Nanoparticle-induced toxicity can be amplified in the pregnant population. A single intranasal administration of titanium dioxide nanoparticles caused no reaction in the non-pregnant. However, the same treatment caused a robust and persistent acute inflammation in pregnant BALB/c mice, as shown by the up regulation of inflammation-associated genes in the lungs (Fedulov et al., 2008 and Lamoureux, et al., 2010).

This inflammation may be partially caused by the suppression of cell-mediated immunity in pregnant females (Weinberg, 1984) . The health of the offspring was also affected by this exposure, as indicated by increased susceptibility to asthma (Fedulov *et al*., 2008). Inhalation and intratracheal instillation of carbon nanoparticles caused pulmonary inflammation in dams and DNA strand breaks in the livers of both dams and offspring (Jackson *et al*., 2012).

The effects on pregnant animals vary with the chemical nature of the nanoparticles.

Nanoparticles might cause toxicity to the embryo by destroying the redox equilibrium in the placenta (Pietroiusti *et al*., 2011), inducing apoptosis in blastocysts (Li *et al*., 2010; Chan and Shiao, 2008), and inhibiting the differentiation of embryonic stem cells (Park *et al*., 2009).

Nanoparticles in maternal circulation use different pathways to enter the offspring, including lactation (Blum *et al*., 2012; Sumner *et al*., 2010 and Gao *et al.,* 2011). Although it is protected by the placental barrier, the fetus is particularly vulnerable. The leakage of nanoparticles across the placenta exposes important organs to nanoparticles and may induce oxidative stress and inflammation in the fetus. Moreover,

maternal inflammatory cytokines induced by nanoparticles can also cross the placenta and affect fetal brain development (Jonakait, 2007 and Meyer *et al*., 2009).

Maternal exposure to nanoparticles may also affect the health of offspring through other mechanisms. For example, exposure of pregnant ICR mice to carbon black nanoparticles induced renal abnormalities similar to tubulointerstitial fibrosis in the kidneys of the offspring (Umezawa *et al*., 2011).

Mechanistically, these changes might be due to the high rate of cell division in the fetus and the immature repair capability for DNA damage. These damages may increase the susceptibility of the offspring to cancer and other diseases (Barton *et al*., 2005).

Titanium (Ti), the ninth most abundant element in the earth's crust, is widely distributed. The average concentration of Ti in the earth's crust is 4400 mg/kg. Owing to its great affinity for oxygen and other elements, Ti does not exist in the metallic state in nature. The most common oxidation state of Ti is +4, but +3 and +2 states also exist. Metallic Ti, TiO_2, and $TiCl_4$ are the compounds most widely used in industry. TiO_2, also known as titanium (IV) oxide, titanic acid anhydride, titania, titanic anhydride, or Ti white, is the naturally occurring oxide of Ti. TiO_2 is a white noncombustible and odorless powder with a molecular weight of 79.9 g/mol, boiling point of 2972°C, melting point of 1843°C, and relative density of 4.26 g/cm3 at 25°C. TiO2 is a poorly soluble particulate that has been widely used as a white pigment (Warheit *et al*., 2007 and Sayes *et al*., 2006).

Approximately four million tons of this pigment are consumed annually worldwide (Ortlieb, 2010). In addition, TiO_2 accounts for 70% of the total production volume of pigments worldwide (Baan *et al*., 2006), and is in the top five NPs used in consumer products (Shukla *et al*., 2011). Metal oxide NPs are of specific interest since some, such as titanium dioxide (TiO_2) , are amongst the most widely used NPs, produced in large volumes, and have been commercially available in several shapes and sizes for decades (Rushton *et al*., 2010 and Xia *et al*., 2013).

TiO_2 ENPs are widely used in products such as cosmetics, clothing, food packaging, drug delivery systems, therapeutics, biosensors, surface cleaning agents, catalysis, etc. since they are transparent and more esthetically pleasing to consumers at this size (Wolf *et al* ., 2003). It can even be used as a pigment to whiten skim milk. TiO_2 NPs are also used in sunscreens (Trouiller *et al*., 2009). In addition, TiO_2 has long been used as a component for articulating prosthetic implants, especially for the hip and knee (Jacobs *et al*., 1991 and Sul, 2010).

Similar to other inorganic NPs, TiO_2 NPs in the systemic circulation has two potential pathways for clearance, i.e., kidneys/urine and bile/feces. The International Program on Chemical Safety (IPCS) for TiO_2shows that most ingested TiO_2 is excreted with urin. Clearance of particles from the liver *via* the bile into the feces is well known in pharmaceutics and is also postulated for TiO_2 NPs (Huggins and Froehlich. 1966).

TiO_2 particles including micro- and nano-sized, are evaluated as a Group 2B carcinogen by WHO/ International Agency for Research on Cancer (IARC) (Baan *et al*., 2006), based on 2-year animal aerosol inhalation studies (Lee *et al*., 1985; Pott and Roller, 2005).

The rapidly developing field of nanotechnology, which is creating materials with size-dependent properties, is likely to become another source of exposure to nanomaterials (Oberdorster *et al* ., 2005).

Thus nanomaterials such as Titanium dioxide TiO_2 should be investigated carefully to evaluate their teratogenic effects .

MATERIALS AND METHODS

Experimental animals

The present experimental study is carried out on the albino rat (*Rattus norvegicus*). The standard guidelines of the Institutional Animal Care and Use Committee (IACUC) were used in handling animals.

Females of 11-13 weeks old were selected for the present study and viginal smears prepared every morning and examined under the light microscope according to the method of Snell (1956) for 5 days to select those in the pro-estrus. Two females with regular estrus cycle were selected in the pro-estrus stage and caged together with one male overnight under controlled environmental conditions of temperature, humidity and light. The first day of gestation was determined by the presence of sperms in the vaginal smear (McClain and Becker, 1975).

A daily record of the weight of the pregnant females was made throughout the whole gestational period. The percentages of abortion were calculated in each group; abortion was determined by the presence of blood drops and sudden drop in the weight of the pregnant females.

Experimental strategy

The TiO_2 nanoparticles used in this study were a kind of nanopowder, anatase , with a particle size of <25nm , purity 99,7% trace metals basis(SIGMA-ALDRICH).

TiO_2 NPs were suspended in distilled water. A Quantitative suspension (0.5mg\kg) of TiO_2 suspended in 1ml distilled water, and then the suspension was ultrasonicated before it was used to treat animals to avoid aggregation and provide an optimum size distribution for dispersed particle agglomerates.

EXPERIMENTAL DESIGN

Route of administration

By intra peritoneal injection.

Time of administration

Scheduled from the 5^{th} day, day after day during both gestation and lactation.

Experimental groups

Group (A): Control group received distilled water from 5^{th} day to the 21^{st} day of lactation.
Group (B): Treated group received 0.5 mg/kg of TiO_2 from 5^{th} day of gestation to the 21^{st} day of lactation.

Developmental observations

On the 20th day of gestation, all pregnant rats of groups (A-B) were sacrificed and total implantation sites, fetal mortality rate (resorped or still birth) and living fetuses were recorded.

On the 7th, 14th and 21st day of lactation respectively the neonates of groups (A-B) were sacrificed.

Fetal body weight, body length, tail length and external malformation were recorded. Head, neck and limbs were examined.

Sample preparation

On the 20^{th} day of gestation, all pregnant rats of groups (A-B) were sacrificed by decapitation.

On the 7^{th}, 14^{th} and 21^{st} day of lactation respectively the neonates of groups (A-B) were sacrificed by decapitation.

The brain was extracted together with parts of liver and kidney to be fixed for histopathological examination.

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Skeletal examination

Fetuses were preserved in 95% ethyl alcohol and were stained with double staining of fetal skeletons for cartilage (Alcian blue) and bone (Alizarin red) according to the method described by Peters (1977).

Oxidative stress investigation

0.2 gm of organ tissue was homogenized in 2ml of phosphate buffer. The homogenate was centrifuged and the clear supernatant was kept in deep freezer at -40◦C for oxidative stress studies.

Determination of Glutathione reduced content

Glutathione content was determined according to the procedure of (Beutler *et al*., 1963). As well as lipid peroxidation.

Statistical analysis

All the values were presented as means (μ) ± standard errors of the means (S.E.M.) Comparison between more than two different groups was carried out using the one-way analysis of variance (ANOVA) followed by Turkey-Kramer's multiple comparison test (Armitage and Berry, 1987), where $P<0.05$ was considered significant. GraphPad Software InStat (version 2) was used to carry out the statistical tests.

RESULTS

Effects of Titanium Dioxide on pregnant albino rats and their fetuses during gestation

Pregnant albino rats

Change in body weight gain

The maternal body weight and weight gain was followed all over the period of gestation for the control and experimental group. The average maternal body weight was recorded on 5^{th} and 19^{th} day of gestation (Table1)**.**

Table 1: Changes in weight gain of pregnant rats during gestational period.

Groups of mothers	Average Wt. of mothers at the 5^{th} day of gestation	Average Wt. of mothers at the 19^{th} day of gestation	Average increase in weight	Percentage of increase
Group A control)(	223.1	268.3	$43.15^{a} \pm 2.043$	20.26%
Group B treated)(	176.85	202.865	$25.955^{b} \pm 0.491$	14.71%

Sample size (n) = 20
Data are represented as mean ± standard error.
Means with the same letter in the same parameter are not significantly different.
F-probability expresses the effect between groups, where $P<0.0001$ is very highly significant.

Effect of TiO_2 on uteri

TiO_2 induced partial resorption and asymmetrical distribution, as well as complete resorption.

Average weight of placenta

The average weight of placenta of pregnant rats treated with 0.5mg/kg of TiO_2 from 5^{th} to 19^{th} day of gestation showed highly significant ($P<0.0001$) decrease as compared to the control group.

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Histological studies of pregnant rats

Examination of serial transverse sections of the brain, liver, kidney of albino pregnant rats treated with TiO_2 on the 20th day of gestation showed some histological changes.

Brain of pregnant rat

The cerebrum showed focal vacuolization and encephalomalacia as well as neuronal degeneration in the hippocampus. While the cerebellum showed degeneration in purkinje cell, vacuolization in white matter and degeneration in granular cell layer.

Liver of pregnant rats

The liver showed fatty change in few hepatocytes with inflammatory cells infiltration in between as well as aggregation of inflammatory cells and cellular pigmentation in portal area.

Kidney of pregnant rats

The kidney revealed some histopathological changes such as vacuolization in lining endothelium of the glomerular with focal inflammatory cells infiltration between the tubules.

Effects of TiO_2 albino rat fetuses during gestation

Fetal resorption

Total resorped fetuses were recorded for control and experimental group. Total rate of resorped fetuses maternally treated with 0.5 mg/kg of TiO_2 was 33.33% compared to the control group.

Growth retardation

The morphological examination of fetuses showed that TiO_2 caused growth retardation represented by decrease in fetal body weight, body length and tail length (table 2).

Table 2: The body weight, body length and tail length of fetuses on the 20th day of gestation.

Groups of fetuses during gestation	Average body wt. of fetuses	Average body length of fetuses	Average body tail length of fetuses
Group A (control)	3.568^{a} ± 0.023	4.683^{a} ± 0.0493	1.387^{a} ± 0.009
Group B (Treated)	1.032^{b} ± 0.055	2.953^{b} ± 0.0531	0.740^{b} ± 0.0215

Data are represented as mean ± standard error.
Means with the same letter in the same parameter are not significantly different.
F-probability expresses the effect between groups, where P<0.0001 is very highly significant.

Morphological malformations

The malformations found in fetuses from treated group were hematoma. The percentage of hematoma in fetuses of control rats is 1.23% while in treated group the percentages of hematoma is 13.2% (fig.1& table 3).

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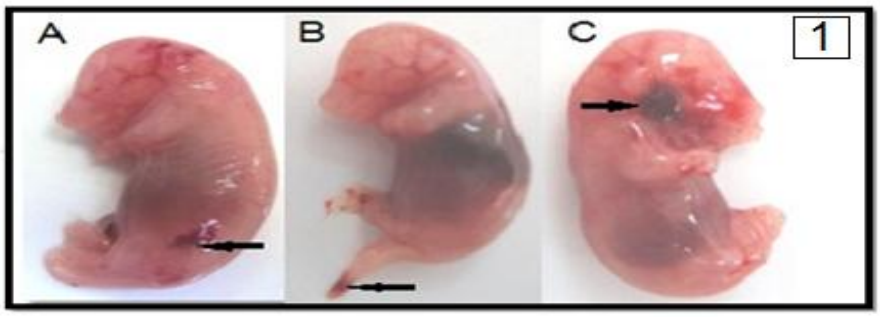

Fig. 1A: A photograph of fetuses on the 20th day of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing hematoma at the hind limb (arrow).
Fig. 2B: A photograph of fetuses on the 20th day of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing hematoma at the tail (arrow) as well as transparence skin.
Fig. 3C: A photograph of fetuses on the 20thday of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing hematoma at the face (arrow) as well as transparence skin.

Table 3: Effect of TiO_2 on the percentage of hematoma in fetuses on 20th day of gestation.

Groups	No. of examind fetuses	No. of hematoma	Percentage of hematoma
Group A (control)	163	2	1.23%
Group B (control)	129	17	13.2%

The data are represented as percentage (%).

Internal anomalies

Observed in treated fetuses were mainly in the form of enlarged liver, growth retardation with visceral hernia and retardation excencephally.

Skeletal examination:

The fetuses of the group maternally treated with 0.5mg/kg of TiO_2 showed lack of ossification of skull, central vertbra, caudal vertebra , tarsus, phalanges and femur and two ribs coming out of the same vertebra and carpus and ulna (fig.2).

Fig.2: A photograph of skeletal system of fetus on the 20thday of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing lack of ossification of skull (red arrow), lack ossification of central vertbra (blue circle), two ribscoming out of the same vertebra (black circle) , non ossification of caudal vertebra (blue circle) and no ossification of tarsus (blue arrow), phalanges (black arrow) and lack ossification of femur (black arrow) .

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Histological studies of fetuses

Examination of serial transverse sections of the brain, liver and kidney of albino rats fetuses maternally treated with TiO_2 on the 20th day of gestation showed some histological changes.

Brain of fetuses

The brain showed vacuolization &degeneration cells in the cerebrum as well as in the cerebellum (fig.3).

Liver of fetuses

The liver showed fatty change in few hepatocytes with dilation in portal vein and inflammatory cells infiltration in portal area (fig.4).

Kidney of fetuse

The Kidney revealed some histological changes such as swelling of glomeruli, and degeneration of lining epithelium of the tubules and swelling of glomeruli, slightly fatty degeneration , pyknotic nuclei and degeneration of lining epithelium of the tubules (fig.5).

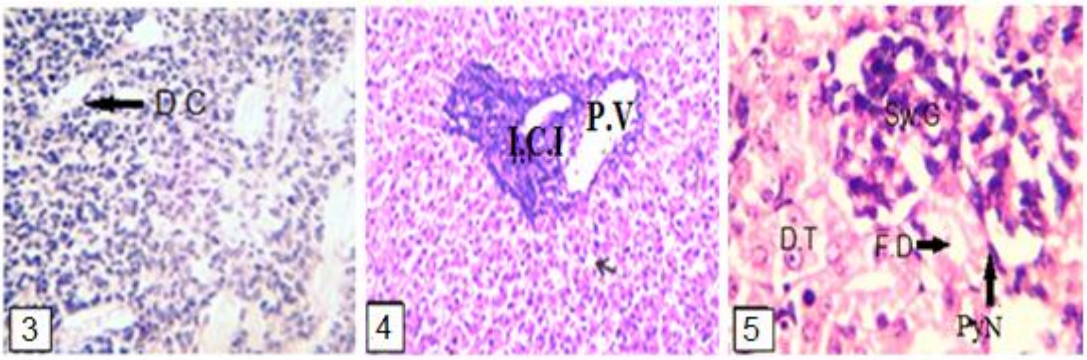

Fig. 3:A photomicrograph of a section of brain of fetus on the 20th day of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing degenerative cell (D.C). H&E 40X

Fig.4 :A photomicrograph of a section of liver of fetus on the 20th day of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing fatty change (arrow) in few hepatocytes with dilation in portal vein (P.V) and inflammatory cells infiltration (I.C.I) in portal area. H&E 40X

Fig.5:A photomicrograph of a section ofkidney of fetus on the 20th day of gestation maternally treated with 0.5mg/Kg TiO_2 from 5th to 19th day of gestation showing swelling of glomeruli (Sw.G), slightly fatty degeneration (F.D), pyknotic nuclei (Py.N) and degeneration of lining epithelium of the tubules (D.T). H&E 200X

Oxidative stress investigations during gestation

Glutathione reduced (GSH) content and Malondialdehyde

The treated rat fetuses on the 20th day of gestation indicated a marked decrease in cerebrum, cerebellum and liver glutathione content throughout the experiment compared to control fetuses and an increase in the lipid peroxidation content.

Effects of TiO_2on albino rat neonates during lactation

Growth retardation

The morphological examination of neonates maternally treated with TiO_2 showed growth retardation represented by decrease in body weight, body length and tail length according to control neonates on the 7th, 14th and 21st day of lactation (table 4).

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Table 4: The body weight, body length and tail length of neonates on 7th, 14th and 21st day of lactation.

Groups During lactation	Average body wt. of neonates			Average body length of neonates			Average tail length of neonates		
	7th day	14th Day	21st day	7th day	14th day	21st day	7th day	14th day	21st day
Group (con)	12.688[a]± 0.053	23.641[a] ± 0.106	39.179[a] ± 0.183	6.852[a]± 0.0107	10.795[a] ± 0.018	13.002[a] ± 0.036	2.626[a]± 0.014	6.447[a]± 0.018	7.161[a]± 0.019
Group (t)	8.227[b] ± 0.111	18.43[b]±0.554	30.917[b]± 0.651	5.870[b]± 0.029	7.754[b]± 0.054	8.974[b]± 0.074	1.991[b]± 0.042	4.014[b] ± 0.054	5.631[b] ± 0.119

Data are expressed as mean ±Standard error
Means with the same latter in the same parameter are not significantly different
F-probability expresses the effect between groups, where P<0.0001 is very highly significant.

Skeletal examination

The group maternally treated with 0.5mg/kg of TiO_2 from 5th day of gestation to 7th day of lactation & to the 14th day of lactation showed two ribs coming out of the same vertebra , absence of left 13th rib and absence of fibula bone.

The group maternally treated with 0.5mg/kg of TiO_2 from 5th day of gestation to 7th, 14th and 21st day of lactation showed absence of left 13th rib.

Histological studies of neonates on 7th, 14th, and 21st day of lactation

Examination of serial transverse sections of the brain, liver and kidney of albino rat neonates maternally treated with TiO_2on the 7th, 14th, and 21st day of lactation showed some histological changes.

Brain

The cerebrum showed degenerated cell as well as degenerated neuron in the hippocampus in those maternally treated till the 7th day of lactation while the cerebellum showed degeneration of purkinje cell layer. While on the 14th day of lactation showed vacuolization in white matter and degeneration cell in molecular layer and neuron in both the cerebrum and the cerebellum (fig.6).

On the 21st day of lactation Pyknotic cells in the cerebrum as well as degenerated cells and focal plaques formation and showing Pyknotic cells in the matrix of striatum in the cerebrum and degenerated cells (fig.7). were also observed.

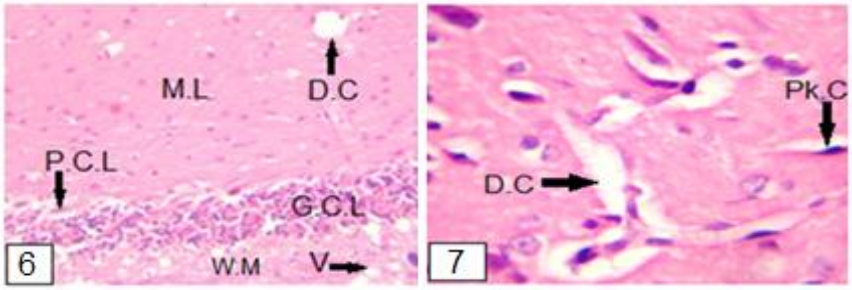

Fig. 6: A photomicrograph of a section of brain of neonates on the 14th day of lactation maternally treated with 0.5mg/Kg TiO_2 from 5th day of gestation to 14th day of lactation showing vacuolization (V) in white matter (W.M) and degeneration cell (D.C) in molecular layer (M.L). H&E 200X

Fig.7:A photomicrograph of a section of brain of neonates on the 21st day of lactation maternally treated with0.5mg/Kg TiO_2 from 5th of gestation to 20th day of lactation showing Pyknotic cell (Pk.C) in the matrix of striatum in the cerebrum (C.S), degeneration cell (D.C). H&E 200X

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Liver of neonates

On the 7th day of lactation, it showed dilation & congestion in the portal vain, degeneration in hepatocytes , as well as inflammatory cells infiltration, odema and dilation of the bile duct (fig.8).

On the 14th day of lactation showed necrosis, fatty change in most of hepatocytes as well as degeneration in hepatocytes and infiltration in the portal area .
On the 21st day of lactation it showed odema in portal area with dilation and congestion in portal vein (fig.9).

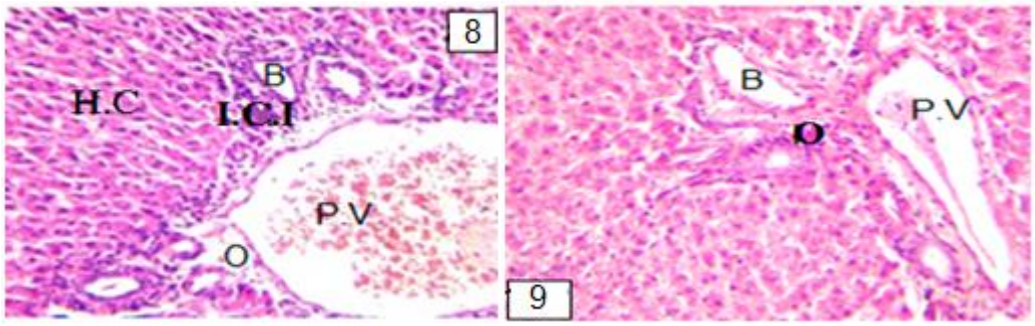

Fig. 8: A photomicrograph of a section of liver of neonates on the 7th day of lactation maternally treated with0.5mg/Kg TiO_2 for 5th to 6th day of lactation showing dilation and congestion in portal vein (P.V) as well as inflammatory cells infiltration (I.C.I), odema (O) and dilation in bile duct (B). H&E 78X

Fig. 9:A photomicrograph of a section of liver of neonates on the 21st day of lactation maternally treated with 0.5mg/Kg TiO_2 from 5th of gestation to 20th day of lactation showing odema (O) in portal area with dilation and congestion in portal vein (P.V). H&E 78X

Kidney of neonates

On the 7th day of lactation the kidney showed destruction tubules, swelling glomeruli, fatty degeneration and pyknotic nuclei.

On the 14th day of lactation it showed proliferation of glomeruli, slight fatty degeneration, hydropic degeneration and proliferation of lining epithelium of the tubules.

On the 21st day of lactation it showed shrinking of glomeruli , slight fatty degeneration degeneration of lining epithelium of the tubules was noticed (fig. 10).

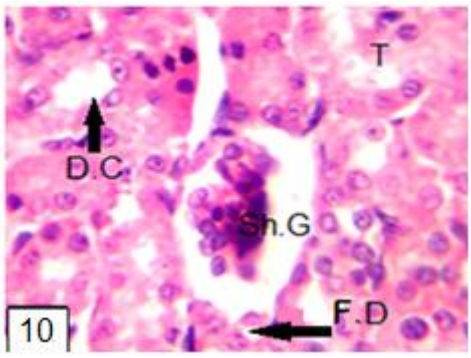

Fig.10: A photomicrograph of a section of kidney of fetus on the 21st day of lactation maternally treated with 0.5mg/Kg TiO_2 from5th of gestation to 20th day of lactation showing shrinking of glomeruli (Sh.G), slight fatty degeneration (F.D), degeneration cell (D.C) and degeneration of lining epithelium of the tubules (D.T). H&E 200X

Oxidative stress investigations during lactation

Glutathione reduced (GSH) content & Malondialdehyde

The treated rat neonates on the 7th, 14th & 21st day of lactation indicated a marked decrease in cerebrum, cerebellum and liver glutathione content and an increase in the lipid peroxidation throughout the experiment as compared to control neonates.

DISCUSSION

Nanotechnology works with substances at nanometer scale, and it offers many solutions for biomedicine .The increased use of nano sized materials in the past several years has compelled the scientific community to investigate the potential hazards of these materials. One of the most widely used nanoparticles is titanium dioxide (TiO_2). Because of its whitening and photocatalytic effects, TiO_2 is widely used in the production of paper, sunscreens, toothpastes, and cosmetics(Wolf *et al*., 2003; Kaida *et al*., 2004; Turkez and Geyikoglu, 2007 ; Wang *et al*., 2008). It is also an important pollutant in car industries (Khezri *et al*., 2012a & 2012b). Moreover, it is used in the environmental decontamination of air, soil, and water (Wang *et al*., 2011). As an ultrafine-sized material, the TiO_2 nanoparticles can enter the human body through various routes such as inhalation, ingestion, and skin (Oberdorster *et al*., 2005 and Jin *et al*., 2008). In recent years, studies have shown that TiO_2 nanoparticles accumulate in the liver, kidneys, spleen, lungs, and heart of animals (Wang *et al*., 2007 & Liu *et al*., 2009).

The aim of the present study was to assess the teratogenic effects of exposure to TiO_2 during pregnancy and lactation on albino rats.

Our data indicated that the intraperitoneal injection of TiO_2 nanoparticles to pregnant and lactating rat mothers impairs many of the morphological and skeletal formation as well as vital organs such as brain, liver and kidney.

TiO_2 induced partial and complete resorption and distribution of fetuses in uterine horns. Also, there was a marked decrease in the average weight of placenta of pregnant rats treated with 0.5mg/kg of TiO_2 from 5th to 19th day of gestation compared to the control groups. In the present study, the morphological examination of fetuses showed that TiO_2 caused growth retardation represented by decrease in fetal body weight, body length and tail length. These finding were in agreement with that of Yamashita *et al*., (2011) who worked on mice and proved that TiO_2 administration results in pregnancy complications. Intraperitoneal injection of TiO_2 resulted in mild effects on the skeletal formation in the developing fetuses and offsprings such as lack of ossification of skull and appearance of two ribs coming out of the same vertebra. There was no data on the effect of TiO_2 on the skeleton on other developing animal models such as mice. The examination of section of the brain of albino rat fetuses as well as neonates treated with TiO_2 showed some histological changes in the cerebrum and cerebellum such as degenerated neurons, vacuolization and focal plaques formation. Our studies revealed that groups of fetuses and neonates maternally treated with TiO_2 showed changes in histological structure of the liver represented by dilation and congestion in portal vain, oedema as well as inflammatory cell infiltration. Similar results were recorded in mice by Chen *et al*., (2009) they prove that maternal exposure to TiO_2 resulted as illustrated in hepatic fibrosis around the central vein where TiO_2 particles attached was extensive , some loss of sinusoid space and hydropic degeneration with minor fatty change. In the current study, administration of TiO_2 resulted in severe histopathological changes in kidney represented by fatty degeneration, shrinking of glomeruli and degeneration of lining epithelium of tubules. These histopathological change of kidney are also found in mice exposed to TiO_2 nanoparticles during pregnancy and lactation (Chen *et al*., 2009). Developing embryos seem to be very sensitive to high levels of ROS, especially during early organogenesis. High levels of oxygen are toxic to the embryo and fetus, apparently due to the fact that the Reactive Oxygen Species (ROS) created in such a condition are excess in relation to the antioxidant capacity of the developing embryos, leading to the production of highly reactive oxygen or nitrogen species and creating oxidative stress and embryonic damage (Ornoy *et al*., 1996 ; Ornoy *et al*., 1999). Our results showed that prenatal exposure to TiO_2 nanoparticles impaired the and oxidative status, causing significant increase in the lipid peroxidation in liver and brain compared to control groups. While significant decrease in glutathione level was recorded in liver and brain of the treated groups. Therefore, it is possible that uncontrolled dispersion of engineered nanoparticles will cause developmental neurotoxicity by

neurobehavioral testing. Our findings suggest the need for great caution to handle the nanomaterials as titanium dioxide especially during pregnancy and lactation.

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