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Protective Role of Purslane Seed Extract on Embryos and Placentae of Rats Treated with Titanium Dioxide Nanoparticles

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Abstract

Background: Titanium dioxide nanoparticles (TiO_2NPs) that are used daily in sunscreens, foods, pharmaceuticals and cosmetics are most prevalent around the world. The potential toxicity of TiO_2NPs on the growth and development of embryo and placenta has been rarely studied during pregnancy. Portulaca oleracea L. (purslane) is a safe daily green vegetable that is considered as a natural source of antioxidants.

Aim of the study: The present study was designed to evaluate the possible protective role of purslane seed extract on the growth and development of embryos and placentae of pregnant rats treated with TiO_2NPs during pregnancy.

Material and methods: Thirty-two pregnant females (12 weeks old,150±200gm) were randomly divided into four groups (8 animals each) as follows:

Group 1 (C): Untreated control

Group 2 (P): Rats treated with purslane seed extract at a dose of 10 mL/kg/day

Group 3 (T): Animals received 0.5 mg/kg/day nano-TiO_2.

Group 4 (P + T): Rats received purslane seed extract at a dose of 10 mL/kg/day followed by TiO_2NPs at a dose of 0.5 mg/kg/day. All treated rats were orally administered from the 6th to the 15th day of gestation. All rats were weighed on the 1st, 6th and 13th day then weighed and sacrificed on the 20th day of gestation. The uteri were removed and weighed. Both implantation sites, live and dead fetuses were counted then the fetuses and placentae were weighed and photographed. Crown rump length and tail length were measured as well as morphological and skeletal abnormalities of live fetuses were examined.

Results: Nano-titanium induced reduction in the weights of pregnant rats, uteri and placentae in addition to fetal growth retardation. Subcutaneous hematoma, as well as skeletal deformities, were observed in fetuses maternally treated with TiO_2NPs. Treatment with purslane seed extract exhibited amelioration in the weights of pregnant rats, uteri, fetuses and placentae as well as in the delayed ossification of the skeleton caused by TiO_2NPs.

Conclusion: Exposure to TiO_2NPs during pregnancy greatly hinders the growth and development of the rat embryos and placentae. Therefore, it is recommended to be very careful while dealing with nanomaterials during pregnancy. It is preferred for eating leafy green vegetables rich in antioxidants during pregnancy.

Keywords: Nanotechnology; Titanium dioxide nanoparticles; Purslane seed extract; Rat embryos; Placenta

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Introduction

The use of nanotechnology and the production of nanoparticles have created new hope for solving human problems [1,2]. Nanoparticles 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 [3,4]. Titanium dioxide nanoparticles (TiO_2NPs) are one of the most highly manufactured and widely used nanoparticles [5]. They are used mainly in paints, coatings, plastics, papers, foods, pharmaceuticals and cosmetics [6,7].

Vegetables are natural sources of antioxidants and other phytochemicals. *Portulaca oleracea* L., commonly known as purslane (P) is extensively used not only as an edible plant but also as a traditional herbal medicine [8]. The plant has muscle relaxant, anticonvulsive, analgesic [9], anti-inflammatory and anti-cancer properties [10]. Purslane is a rich source of omega-3-fatty acids, α-tocopherols, ascorbic acid, β-carotene and glutathione. Its seeds also contain a high percentage of α-linolenic acid [11,12].

The placenta is a unique temporary organ vital for the development

of mammals and plays many roles, including endocrine, nutritional and barrier functions. Also, the placenta becomes a target for drug-or chemical-induced adverse effects.

Aim

The main aim of the study is to evaluate the possible protective role of purslane aqueous seed extract on the growth and development of embryos and placentae of pregnant rats treated with TiO_2NPs during pregnancy.

Materials and Methods

Chemicals

Titanium dioxide nanoparticles: Titanium dioxide nanoparticles used in this study were a kind of nano powder, anatase, with a particle size of <25 nm, purity 99.7% trace metals basis (SIGMA-ALDRICH). The TiO_2NPs were suspended in ultrapure water (Promega, Madison, WI, USA) at a concentration of 20 mg/ml as a stock solution. The stock solution was dispersed by an ultrasonic vibrator for 30 min, after which the suspension was diluted in 1× Holt buffer (60 mmol/L NaCl, 0.67 mmol/L KCl, 0.3 mmol/L $NaHCO_3$, 0.9 mmol/L $CaCl_2$, pH 7.2) to a working concentration of 0.5 mg/L [13]. Titanium dioxide nanoparticles were orally administered at a dose of 0.5 mg/kg/day from day 6 up to day 15 of gestation.

Purslane seed extract: Purslane seed were purchased from the seed seller, Cairo, Egypt. One liter of boiled distilled H_2O was added to 100 gm. of grinded purslane seeds, cooled and filtered. The concentrated yield extract was diluted with distilled H_2O for the desired volume (1:10 wt/vol.) [14]. Purslane seed extract was administered at a dose of 10 mL/kg/day by an oral gavage from day 6 up to day 15 of gestation.

Animals

Ten mature fertile male and 50 virgin female albino rats (12 weeks old, 150 ± 200 gm) were obtained from the Laboratory Animal Colony, Helwan. Animals were housed in especially designed cages, eight females per cage. The males were kept separated from females until mating. All rats were kept under strict care and hygienic conditions of temperature, relative humidity and a 12-hr. light/dark cycle. They were fed on the diet from the Factory of Oil and Soap Company, Cairo, Egypt. Drinking tap water and food pellets as well as some vegetables as a source of vitamins were available *ad libitum*. Prior to dosing, the rats were acclimated to the laboratory environment for one week then female rats were mated overnight (each male rat was mated with 2 females). Vaginal smears were checked in the next morning for finding a vaginal plug and that day was considered as gestational day (GD) 1.

Experimental design

Thirty-two pregnant females were randomly divided into four groups (8 animals each) as follows:

Group 1 (C): Untreated control in which pregnant rats fed on normal diet.

Group 2 (P): Rats treated with aqueous purslane seed extract at a dose of 10 mL/kg/day.

Group 3 (T): Animals received 0.5 mg/kg/day nano-TiO_2.

Group 4 (P + T): Rats received purslane seed extract at a dose of 10 mL/kg followed by TiO_2NPs at a dose of 0.5 mg/kg/day.

All treated rats were administered by an oral gavage from the 6th to the 15th day of gestation. All rats were weighed on the 1st, 6th and 13th day then weighed and sacrificed on the 20th day of gestation. The intact uteri with their ovaries attached to them were quickly removed, weighted and photographed. Number of implantation sites live, and dead fetuses were counted and recorded for each litter. The fetuses (live, dead or resorbed) and placentae were then detached and photographed. The placentae and live fetuses were weighed, and fetal external morphological abnormalities were examined. Crown rump length and caudal length of live fetuses were measured. The maternal liver tissue was homogenized for analysis of GSH, MDA contents and SOD activity.

Evaluation of fetal skeleton development

Two live fetuses from each pregnant rat were fixed in 95% alcohol for 7 days. Then skinned, eviscerated and stained with alizarin red S and alcian blue double staining (Salaramoli et al., 2015). The fetal skeletons were examined under the stereo microscope.

Statistical analysis

The obtained data were analyzed using the one-way analysis of variance (ANOVA) followed by Post HOC tests (LSD) analysis to compare all treated groups with control then compare various groups with each other. Results were expressed as mean ± standard error (SE) [16].

Results

General condition of pregnant rats

All control pregnant rats were mostly active during the day, calm by night time, had good appetite and kept their fur clean. Rats treated with P extract appeared more or less similar to control. After administration of T from GD 6 up to 15, the exposed rats became inactive and exhibited salivation, emaciation, anorexia, prostration and alopecia. Pregnant rats treated with P extract followed by T exhibited anorexia throughout the administration period. No abortions were recorded were recorded in all groups.

Maternal body weight change

In the present study, the changes in body weight of control and treated rats that recorded on the 1st, 6th, 13th and 20th days of gestation are shown in the table (Table 1).

The data indicated that, the pregnant rats of both the control and all experimental groups showed non-significant increase in the body weight change during the 1st week of gestation. However, a steady increase in the body weight change from 1st to 13th days of gestation is recorded. From day 6 up to day 13 of gestation, the mean values of maternal body weight change were 28.0±2.2 in control, 27.5±3.6 in P extract, 23.4±1.1 in T and 24.5±1.8 in P+T treated groups. From day 13 to day 20 of gestation, the average body weight change of control and P extract groups continued to increase approximately at the same rate while, those of T and P+T treated groups showed non-significant change compared to control group. The percentage of change reached to -12.903% in T and -6.45% in P+T experimental groups compared to control. From day one up to day 20 of gestation, there was no statistically significant difference in maternal body weight change between the control and P extract groups. However, the change of T and P+T (78.0±2.8) treated groups was significantly ($P \leq 0.05$) lower than that of the control. The percentage of change decreased to -17.41% in T and -12.95% in P+T groups.

Table 1: Maternal body weight change (gm) of control and different treated groups through the experimental period.

Weight	Body weight change (gm)									
	Days 1-6	% of Change	Days 6-13	% of Change	Days 13-20	% of Change	Days 1-20	% of Change	Corrected maternal body weight change	Net weight gain
Groups	Mean ± S.E		Mean ± S.E		Mean ± S.E		Mean ± S.E		Mean ± S.E	Mean ± S.E
Control	26.5±3.1	0	28.0±2.2	0	31.0±1.9	0	89.6±1.6 b	0	24.9±3.6	-3.12±5.0
P	25.0±2.8	-5.66	27.5±3.6	-1.79	30.9±2.8	-0.32	84.2±2.9 b	-6.03	23.9±2.4	-3.6±5.2
T	20.0±1.7	-24.53	23.4±1.1	-16.43	27.0±1.6	-12.903	74.0±4.2 a	-17.41	22.0±4.8	-1.4±5.7
P+T	22.0±2.3	-16.98	24.5±1.8	-12.5	29.0±1.8	-6.45	78.0±2.8 a	-12.95	24.9±5.0	0.4±6.5
Significance between groups	N.S		N.S		N.S		P≤0.01		N.S	N.S
F- value	1.4		0.9		0.8		5.2		0.11	0.1

Where: All results represent Mean ± SE of 8 animals.

aSignificant when compared to control group ($P \leq 0.05$).

bSignificant when compared to treated groups ($P \leq 0.05$). Means, which have the same superscript symbol (N.S.), are not significantly different.

Where: P = Portulaca; T = Titanium; P+T= Portulaca+ Titanium

Average weight of gravid uteri

The average weights of uteri from control and treated pregnant rats are recorded in the table 2 (Table 2).

The average weight of uteri from pregnant rats treated with P extract showed non statistically significant difference ($p \leq 0.05$) as compared to control. On contrast, the average weight of uteri of pregnant rats treated with T and P+T from the 6th to the 15th day of gestation exhibited significant ($p \leq 0.05$) decreases than those of control and P extract groups.

Survival and mortality of fetuses

The total prenatal mortality rate was represented by fetal resorptions and stillbirths (dead fetuses). On the 20th day of gestation, the number of implantation sites, live, dead (or resorbed) fetuses were counted and are shown in table 3 (Table 3).

Administration of P extract had no significant effect on the number of implantation sites (8±0.60) in comparison with control (9±0.3) group. Significant ($P \leq 0.05$) decreases in the number of implantation sites were recorded in T (6±0.5) and P+T (7±0.4) treated groups.

There were no dead or resorbed fetuses in the control and P extract groups, while the mean number of dead fetuses maternally treated with T was significantly higher ($p \leq 0.01$) than that of the control group. The number of dead fetuses of P+T treated group showed no significant change in comparison with control dams.

Compared with the control, the mean number of live fetuses in P extract group detected no significant change. However, significant ($p \leq 0.05$) decreases in the number of live fetuses of nano-TiO_2 (5±0.4) and P+T (7±0.5) treated groups was recorded in comparison with the control.

Average weights of placentae and live fetuses

The average weights of placentae and live fetuses are recorded in the below table 4 (Table 4).

There is no significant difference between the P extract treated and control groups for placental and fetal weights. In addition, non-significant decrease in the average weights of placentae is detected in T treated group compared to the control. This weight is improved in P+T that reached the mean value of control group.

Table 2: Average gravid uterine weights (gm) of control and different treated groups.

Groups	Uterine weight (gm)
	Mean ± S.E
Control	36.8±2.8
% of change	0
P	32.8±2.4
% of change	-10.87
T	28.6±2.3
% of change	-22.28
P+T	28.6±3.1
% of change	-22.28
Significance between groups	N.S
F- value	2.1

Where: All results represent Mean ± SE of 8 animals.

[a]Significant when compared to control group ($P \leq 0.05$).

[b]Significant when compared to treatment group ($P \leq 0.05$). Means, which have the same superscript.

Symbol (N.S.), are not significantly different.

Where: P=Portulaca; T=Titanium; P+T=Portulaca+Titanium.

Table 3: The mean number of implantation sites, live and dead or resorbed fetuses obtained on day 20 of gestation from control and different treated groups.

Groups	Number of implantation sites	Number of live fetuses	Number of dead or resorbed fetuses
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Control	9±0.3	9±0.3	0±0.0
	0	0	0
P	8±0.60	8±0.6	0±0.0
% change	-11.11	-11.11	
T	6±0.5	5±0.4	1±0.2
% change	-33.33	-44.44	
P+T	7±0.4	7±0.5	0±0.0
% change	-22.22	-22.22	
Significance between groups	P≤0.01	N.S	N.S
F- value	8.5	17.2	28

Where: All results represent Mean ± SE of 8 animals.

[a]Significant when compared to control group ($P \leq 0.05$).

[b]Significant when compared to treated group ($P \leq 0.05$). Means, which have the same superscript

Symbol (N.S.), are not significantly different.

Where: P=Portulaca. T=Titanium. P+T= Portulaca+Titanium

Fetal growth retardation represented by a significant decrease ($p \leq 0.05$) in the fetal weight in T and P+T treated groups as compared to the control. The placental coefficient recorded no significant change and all experimental groups.

Crown rump length and tail length of live fetuses

The mean values of fetal crown rump and tail lengths on the 20th day of gestation are represented in the below table 5 (Table 5).

Treatment with P extract exhibited no significant difference in the fetal crown rump and tail lengths compared to the control.

Fetal growth retardation was indicated by a significant decrease ($p \leq 0.05$) in the average values of fetal crown rump and tail lengths of T treated group in comparison with control. The percentage of change decreased to -10.69% and -18.10% respectively compared to the control.

Compared with the control, both fetal crown rump and caudal lengths of P+T treated group recorded significant ($P \leq 0.05$) decreases.

External morphological studies

The fetuses from control (Plate 1A) and P (Plate 1B) treated rats appeared with normal shape, normal shape and size. Maternal administration of T caused growth retardation represented by improvement in growth of fetuses maternally treated with P extract followed by T (Plate 1D) was demonstrated despite skin dryness.

Gestational exposure to T showed subcutaneous hematoma in some fetuses in head, limbs and tail regions. The least mean value of hematoma was found in control followed by P extract group with no significant change. However, fetal rats maternally treated with T revealed highly significant ($P \leq 0.01$) increase in the mean value of hematoma with a percentage of change 576% as compared to the control group. Moreover, treatment with P+T lowered the mean value of hematoma caused by T. This value was statistically non- significant compared to control (Table 6).

Skeletal studies of fetuses

The bony parts of the skeleton appeared red in color while the cartilaginous parts appeared blue in color. Ossification process has been obviously completed in both control (Plate 2A) fetuses and maternally treated with P extract (Plate 2B). On contrast, fetuses from dams treated with T showed severe lack of ossification (Plate 2C) however, this lack is markedly improved in P+T (Plate 2D) treated group.

Table 4: Mean weights of placentae and embryos (gm) obtained on day 20 of gestation from control and different treated groups.

Groups	Placental weight (gm)	Fetal weight (gm)	Placental Coefficient
	Mean ± S.E	Mean ± S.E	Mean ± S.E
Control	0.38±0.04	2.51±0.14	0.15±0.01
% of change	0	0	0
P	0.39±0.02	2.56±0.07	0.15±0.01
% of change	2.63	1.99	0
T	0.35±0.04	2.00±0.14	0.18±0.02
% of change	-7.89	-20.31	20
P+T	0.38±0.03	2.22±0.07	0.17±0.01
% of change	0	-11.55	13.33
Significance between groups	N.S	P≤0.01	N.S
F- value	0.33	5.3	0.78

Where: All results represent Mean ± SE of 8 animals.

[a]Significant when compared to control group ($P \leq 0.05$).

[b]Significant when compared to treated groups ($P \leq 0.05$). Means, which have the same superscript symbol (N.S.), are not significantly different.

Where: P = Portulaca; T= Titanium; P+T= Portulaca+Titanium

Table 5: Crown rump length and tail length of fetal rats on day 20 of gestation from control and different treated groups:

Groups	Crown rump length (cm)	Tail length (cm)
	Mean ± S.E	Mean ± S.E
Control	3.18±0.07b	1.16±0.02b
% of change	0	0
P	3.11±0.03b	1.16±0.03b
% of change	-2.201	0
T	2.84±0.05a	0.95±0.02a
% of change	-10.69	-18.1
P+T	3.02±0.06a,b	1.14±0.03b
% of change	-5.03	-1.72
Significance between groups	P≤0.001	P≤0.001
F- value	7.3	17.9

Where: All results represent Mean ± SE of 8 animals.

[a]Significant when compared to control group ($P \leq 0.05$).

[b]Significant when compared to treated groups ($P \leq 0.05$). Means, which have the same superscript symbol (N.S.), are not significantly different.

Where: P = Portulaca; T= Titanium; P+T= Portulaca+Titanium.

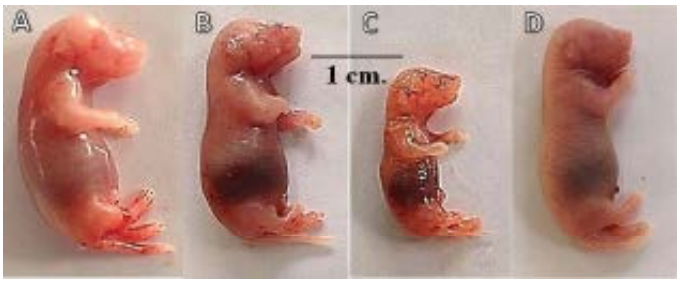

Plate 1: Photographs of fetuses on day 20 of gestation from control (A) P extract (B) Notice the very small growth retarded fetus of T group (C) Also notice the marked improvement in fetal growth of P+T (D) Improvement of growth despite skin dryness of a fetus maternally treated with P extract followed by TiO_2NPs.

Table 6: Effect of T on the hematoma of fetuses.

Groups	Hematoma
	Mean ± S.E
Control	0.50±0.19[b]
% of change	0
P	1.25±0.31[b]
% of change	150
T	3.38±0.50[a]
% of change	576
P+T	1.75±0.94
% of change	250
Significance between groups	P≤0.01
F- value	4.6

Where: All results represent Mean ± SE of 8 animals.

[a]Significant when compared to control group ($P \leq 0.05$).

[b]Significant when compared to treated groups ($P \leq 0.05$). Means, which have the same superscript symbol (N.S.), are not significantly different.

Where: P=Portulaca; T=Titanium; P+T=Portulaca+Titanium

Skeletal variations

Administration of T to pregnant rats produced a variety of rib abnormalities in the surviving fetuses such as irregular, wavy,

amorphous, angulated, forked and reduced ribs. None of the previous abnormalities were shown in fetuses of the control and P extract groups. Moreover, administration of P+T revealed normal appearance and good ossification of the ribs (Plate 3).

Fetal rats maternally treated with T also exhibited less ossified thoracic vertebrae with forked centra of the 2nd and 3rd lumbar vertebrae in addition to incomplete development of the rib which coalesce with the vertebrae (Plate 3).

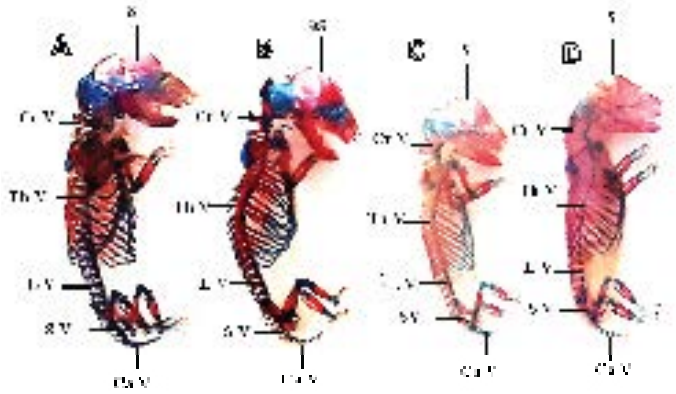

Plate 2: Photograph of the skeletal systems of 20 days old fetuses from control and treated pregnant rats. (A and B) Skeleton of fetuses from control and maternally treated with P extract showing well ossified skull, vertebral column, fore and hind limbs. (C) Skeleton of fetus maternally treated with T from day 6 up to day 15 of gestation exhibiting dysplasiaof the skeleton in addition to (D) partial amelioration of ossification of a skeleton of fetus maternally treated with P+T (D). Alizarin red S and alcian blue double staining, X 2.4.
Where: S=Skull, Cr V=Cervical Vertebrae; Th V=Thoracic vertebrae; LV= Lumbar vertebrae; S V= Sacral vertebrae and Ca V=Caudal vertebrae.

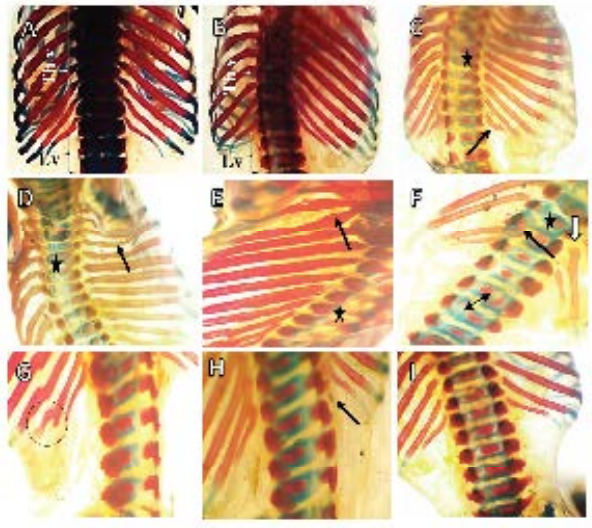

Plate 3: Photographs of the ribs, thoracic and lumbar vertebrae of fetuses on the 20th day of gestation. (A and B) The ribs and thoracic vertebrae of control and and P extract groups showing normal bone ossification. (C) Very weak ossification with curved last rib (arrow), (C, D, E and F) incomplete ossification of thoracic and lumbar vertebral centra (star) and (D) curved 4th rib (arrow). (E) amorphous rib (arrow), (F) bipartite vertebral centra of the 2nd and 3rd lumbar vertebrae (double arrow), costal separation of the ribs (number 12 and 13) (white arrow), angulated rib (black arrow) as well as (G) forked (circle) and (H) rudimentary (arrow) ribs are observed. (I) Partial amelioration of ossification of ribs in addition to thoracic and lumbar vertebrae of a fetus maternally treated with P+T are observed. Alizarin red Sand alcian blue double staining, X10.
Where: Th v = thoracic vertebrae. Lv = lumbar vertebrae.

Table 7: Effects of T on superoxide dismutase (SOD) activity, reduced glutathione (GSH) and malondialdehyde (MDA) contents of maternal liver tissue of control and different treated rat groups

Groups	SOD u/gm tissue	GSH mg/gm tissue	MDA µm/gm tissue
	Mean±S.E	Mean±S.E	Mean±S.E
Control	14.5±0.50	124.1±3.68	46.3±2.30
% of change	0	0	0
P	12.3±1.20	115.4±5.78	101.9±13.56
% of change	-15.17241379	-7.010475423	120.0863931
T	5.4±0.62	79.5±7.89	127.3±16.40
% of change	-62.75862069	-35.93875907	174.9460043
P+T	13.0±0.50	117.5±6.76	109.0±4.00
% of change	-10.34482759	-5.3182917	135.4211663
Significance between groups	P≤0.001	P≤0.001	P≤0.001
F- value	27.9	10.4	10.3

All results represent Mean±SE of 8 animals.
aSignificant when compared to control group ($P \leq 0.05$).
b Significant when compared to treated groups ($P \leq 0.05$). Means, which have the same superscript symbol (N.S.), are not significantly different.
Where: P = Portulaca.T= Titanium.P+T= Portulaca + titanium.

Discussion

Titanium dioxide nanoparticles are one of the most prevalent exposure scenarios because humans are frequently exposed to TiO_2NPs contained in food products, liquid beverages and drugs [17,18]. The molecular mechanism of TiO_2 nanoparticle- induced toxicity is regarded as the induction of inflammation and generation of ROS and cell proliferation [19].

The placenta is an important link between the mother and the fetus and has substance transport, barrier and endocrine functions [20]. The toxicity of different types of nano-TiO_2, including damage to the liver, lung, kidney, spleen, heart, brain, testis and ovary of mice or rats [21], stomach and thymus of mice [22,23] and female reproductive system injury [24]. However, the evidence for placental developmental toxicity caused by nano-TiO_2 in mammals was limited. The current study was conducted to evaluate the possible protective role of P seed extract on the growth and development of rat embryos and their placentae maternally treated with TiO_2NPs during pregnancy.

The present results demonstrated that, maternal exposure to 0.5 mg/kg/day TiO_2NPs resulting in emaciation, anorexia, prostration and alopecia. This may be due to damaging the epithelial cells of the digestive tract; malabsorption and all these effects can cause the reduction of the body weight. On contrast, Lee J, et al. (2019) found that, oral exposure of TiO_2NPs to pregnant rats had no marked toxicities in terms of general clinical signs [25].

Maternal exposure to 0.5 mg/kg/day of TiO_2NPs markedly reduced the maternal body weight, uterine weight, while, increased the number of both dead and resorbed fetuses as well as fetal growth retardation. These findings are similar to the findings approved by Yamashita et al. (2011), EL-Ghareeb A, et al. (2015) and Hong FS, et al. (2017d) [20,26,27]. These changes may be due to pregnancy complications after administration of T to mice [26] or due to damage caused by OS induced by T exposure to embryos [28]. In addition, Hong FS, et al. (2017d) reported that, nano-TiO_2 content in the maternal blood was negatively correlated with fetal weight. Placenta and embryo may be the second target organs of nano-TiO_2 toxicity, and the abnormal development of placenta may be one of the causes of nano-TiO_2-induced fetal growth retardation. On contrast, Lee J, et al. (2019) found that, there were no marked toxicities in terms of body weight, uterine

weight, fetal and placental weights [25].

Furthermore, no significant change in the uterine weight between control and P extract groups was observed. This may be due to the absence of estrogenic substance in the extract [29], since the estrogenic substance increases the wet weight of uterus [30].

In the present work, the cartilaginous and bony parts of the skeleton of control and P extract groups showed uniform staining on day 20 of gestation. However, skeletons of fetuses maternally treated with T resulted in severe lack of ossification and absence of sternum and ossification centers of some bones as well as skeletal deformities of the vertebrae, ribs and limbs in comparison with control group and P extract groups. These results are in accordance with Zhang and Wang (2004) who reported that, T inhibited bone Ca deposition and may lead to the formation of a barrier in the ossification center during bone development [31]. Furthermore, damage to osteoblasts and chondrocytes and increased osteoclast activity may result in an increase in bone Ca dissolution [32,33]. Moreover, Hong FS, et al. (2017c) demonstrated that, oral administration of 100mg/kg TiO_2NPs to pregnant mice can cross the blood fetal and placental barriers and suppresses embryonic development and induces fetal skeletal malformation. The affected bone ossification may have been due to TiO_2NPs interfering with the permeability of Ca^{2+} in the placenta and caused deformities including rib and sternum absence. In addition, T treatment significantly reduced the ratio of placenta/fetal weight in mice on GD 13. In the present study, TiO_2NPs increased MDA content of maternal liver tissue compared to the control. This result agrees with the results of Eid RS, et al. (2018) and Karimipour M, et al. (2018) [34,35]. This may be due to OS induced by TiO_2NPs exposure and liberation of free radicals. Purslane detected mild amelioration despite non- significant decrease in MDA content which increased by T. This result is consistent with the result of Ahangarpour A, et al. (2016) who reported that, purslane decreased MDA contents in uterus and ovarian tissues of aging female mice due to the antioxidant activity of purslane [36]. Antioxidants work by neutralizing or scavenging free radicals by hydrogen donation before they can attack cells and other biological components) [37]. Purslane has the propensity to attenuate OS by reversing the inhibition of Na+/K+ ATPase activity [38].

In the present results, gestational exposure to TiO_2NPs decreased SOD activity and GSH content in comparison with the control. These results are in accordance with the results of Eid RS, et al. (2018) [34]. The decrease in the antioxidant enzymes may be due to induction of hepatic toxicity by nano-TiO_2 through increasing OS and liberation of free radicals. In addition, the decreased activity of SOD in liver tissue of zebra fish after T exposure [39] may be due to reflecting the balance of oxidation/antioxidant system in organisms [40].

On contrast, P extract increased SOD activity and GSH content in comparison with the control. These results agree with the results of Zidan Y, et al. (2016) who recorded that, purslane is an excellent source of antioxidants, vitamins, α-tocopherol, ascorbic acid and β-carotene as well as glutathione [41]. Moreover, high SOD activity was believed to be due to increased dismutation of superoxide anions due to their production.

Conclusion

In the current study, gestational exposure to TiO_2NPs in rats caused fetal growth retardation and impaired development of placenta. Fetal growth retardation and placental toxicity may be due to TiO_2NPs accumulation in placenta and fetus. Therefore, it is recommended to be very careful while dealing with nanomaterials and it is preferred for eating leafy green vegetables rich in antioxidants during pregnancy.

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