Toxic Impact of Titanium Dioxide (TiO_2) In Male Albino Rats with Special Reference to its Effect on Reproductive System

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Abstract: The present study was directed to explore the toxic effects of orally administered TiO_2 in mature male albino rats . Eighteen mature male albino rats were classified into three equal groups. The first group was used as control and fed on TiO_2 free ration (C), the second and the third groups (T1) and (T2) were fed on ration containing 1% and 2% TiO_2 respectively for 65days . The body weight of male albino rats fed 1% and 2% TiO2 showed a significant decrease along the experimental period. Animals were scarified after termination of the experimental period. The sera were separated for estimation of nitric oxide and testosterone levels. Liver samples were preserved for antioxidants enzyme activities determination. Liver, testes and seminal vesicle samples were preserved in formalin for histopathological study. The results indicated that TiO_2 resulted in a significant decrease in body weight gain, sperm motility %, sperm cell concentration, sperm viability and serum testosterone level. While, a significant increase in sperm abnormalities, serum nitric oxide (NO), hepatic superoxide dismutase (SOD), glutathione reductase (GR) enzyme activities and malondialdehyde (MDA) concentration were recorded. Histopathological findings revealed reduction in the number and size of the epithelial lining of the tubuloalveolar gland and hyperplastic glandular epithelium of seminal vesicle. Testes showed mild spermatogenesis besides congested testicular blood vessels. Liver showing vacuolar, hydropic degeneration and cell death of some hepatic cells and steatosis .The present study concluded that, TiO_2 elicited a marked ruinous effect on male fertility and biochemical parameters as well as histopathological picture.
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1. Introduction:

Colors have been used to make food more attractive and appetizing for centuries, In Egyptian Tombs dating as far back as 1500 B. C depict the making of colored candy (Marmion, 1987). Several synthetic food colors used in food industry proved numerous side effects such as uticaria (Chafee and Settipane, 1967), genotoxic effects (Combes and Haveland-Smith, 1982), endocrinal disturbances (Jennings et al., 1990), behavioral disorders (Pollock and Warner, 1990), and neurological effects (Tanaka, 2001).

Titanium is one of the eight most abundant in the earth's crust and consequently enters the food chain to some degree. Human are estimated to consume approximately 300g/titanium/day in food (Dunford et al., 1997). Moreover, TiO2 accounts for about 70% of the total volume of pigment production world wide (Bann et al., 2006).

The Federal Regulations of US Government limit usage of TiO2 in food products to 1% by weight (Wang et al., 2007). Oral route is a potential exposure route for general population due to TiO2 used as white pigment on tooth paste, drug capsule (Baan et al., 2006), in tableted drug products (Ghoropade et al., 1995), in dairy based products as a whitener in manufacture of different types of cheese (Leone, 1973), dairy based drinks, chocolate, milk, coca, soybean products, milk powder, margarine, processed meat, table and soda water, sausage casing (JECFA, 2006), in bread flour and in the confectionary (Lorenz and Maga, 1973). Also, TiO2 therapeutically used in sunscreens and cosmetic creams (Gelis et al., 2003). There have been a relatively few systematic studies that have employed pigmentary TiO2 (Bermudez et al., 2002). Most studies on TiO2 toxicity in mammals were focused on the pulmonary impact of inhaled or dermal exposure (Wang et el., 2007). Mahrousa (2004) reported that 4mg/kg body weight of TiO2 for 90 days in rates resulted in non significant change in DNA, and RNA content in liver and testis. Schapiro et al., (1995) reported that there were numerous studies shown that TiO2 significant increase the production of hydroxyl radicals. Guo et al. (2009) studied the effect of nanosized TiO2 (200 and 500 mg/kg) in male ICR mice aged 6 weeks injected intraperitoneally ever other day for five times. One week after drug cessation, low dose group

showed non significant changes, while high dose group exhibited a significant increase in serum ALT, ALT/AST ration and BUN. Furthermore, reduction in sperm density, motility and increased sperm abnormalities with germ cell apoptosis concomitant with no obvious pathological changes in liver, kidney, spleen, testis and epididymis.

It is pertinent to record that paucity of information concerning the reproductive study on TiO2. Thus this study was carried out to investigation the oral toxic impact of titanium dioxide in male albino rats with special reference to its effect on fertility.

2. Materials and methods

Experimental Animals

Eighteen mature male albino rats weighing 180- 200g were used. The animals were obtained from Faculty of Veterinary Medicine, Zagazig University (laboratory animal's housing unit). Animals were clinically healthy, kept under hygienic condition, housed in metal cages with hard wood shavings as bedding. They were maintained on basal ration and given water *ad-libitum* for two weeks of acclimatization before use.

Chemical

Titanium dioxide (TiO_2): It is manufactured by Riedel- deHaen and was obtained by Sigma-Aldrich Laborchemikalien GmbH. TiO_2 was added to the ration at level of 1% and 2% according to *Ghoropade et al., (1995*).

Description

It is a white odorless powder with molecular weight 79.88 g/mole

Solubility:-It is insoluble in water and other solvents. It is dissolved slowly in hydrofluoric acid and in hot concentrated sulphuric acid. Water soluble matter not more than 0.5%, some preparations can be made hydrophilic by suitable surface treatment.

Synonyms

Titanium dioxide, titanium peroxide, Titania, anatase, cosmetic white, Tipaque, titanium oxide, pigment white 6, titanium white, E 171, Tania.

Methods

Rats were randomly distributed into three groups each of six. The first group control group (C) fed on TiO_2 free ration. The second and third groups fed on ration containing 1% and 2%TiO_2 (T1&T2) respectively for 65 days according to (Wang et al., 2007). All rats were weighed before the start of the experiment (preliminary weights). Rats were scarified at the end of the experimental period. Serum samples were collected and kept at -20°C for biochemical studies. For seminal picture; The cauda epididymis were minced in normal saline and a drop of this epididymal suspension was picked up for seminal analysis and recording the epididymal spermatozoal characters (Hafez, 1970), sperm motility (Slott et al., 1991), sperm cell concentration per ml of semen (Robb et al., 1987), sperm abnormalities and live % of spermatozoa (Filler, 1993). Serum testosterone was determined according to Wilson and Foster, (1992) using testosterone kit (Egyptian Co. of chemicals) which depend on the method of enzyme immunoassay. Serum concentrations of nitrite according to Torre et al., (1996) by Griess reaction, superoxide dismutase (SOD) activity was assayed by *Niskikimi* et al., (1972), glutathione reductase (GR) activity was determined by Beutler, (1975) and Malondialdehyde (MDA) concentration according to Draper and Hardly, (1990), using Shimudzu type spectrophotometer manufactured by Incorporation Kyoto, Japan. Testis, seminal vesicle and liver were fixed in 10% formalin for histopathological examination according to Bancroft et al., (1996). Statistical analysis of data was assessed according to SPSS, (1997).

3. Results

Clinical signs

Clinical signs showed depression, anorexia and white feces among the different dose levels of TiO_2 treated male rats along the experimental period. Moreover, addition of TiO_2 either 1% or 2% for 65 days of feeding, significantly decrease ($P< 0.05$) the body weight compared to the control (Table 1).

Effects of TiO_2 on male fertility

Concerning the sperm motility; there was non significant decrease in sperm motility percentage of male albino rats fed on low level of TiO_2, while a significant decrease ($P< 0.05$) was recorded in high level fed group compared with the control. The mean values of sperm cell concentration recorded a significant decrease ($P< 0.05$) in both treated groups comparing with the control. There was a significant increase in percentage of sperm abnormalities which was dose dependent and compared to the control one. Results are depicated in Table 2 (Fig 1-II, Fig 1-III A, B, C, D). Regarding sperm viability showed that 1% and 2% TiO_2 caused a significant decrease ($P< 0.05$)comparing with the control group .The testosterone level recorded a significant decrease ($P< 0.05$) in both TiO_2 treated groups compared to control (Table 2).

Biochemical parameters

TiO_2 1% and 2% resulted in a significant increase ($P< 0.05$) in NO production, SOD and GR

enzyme activities compared with control. MDA showed non significant increase in TiO_2 treated groups (Table 3).

Post mortem changes

Macroscopically; seminal vesicles of TiO_2 treated male rats showed hypertrophy in low dose level, while atrophy in high level (Fig 2-A).

Histopathological findings

The epithelial lining of the seminal vesicle showed hyperplastic changes with little or absence of secretion beside edema of trabiculae observed in all rats fed 1% TiO_2 (Fig. 2-C), while there was reduction in number and size of the epithelial cell lining of the tubuloalveolar glands in rats fed 2% TiO_2 (Fig 2-D).The seminiferous tubules revealed mild spermatogenesis with congested interstitial blood vessel with endotheliosis in T1 (Fig. 3-B). Thickened tunica albuginea with degenerated spermatogonial cell layers and spermatocytes with absence of spermatogenesis were also seen particularly in rats fed 2% TiO_2. The hepatocytes suffered from various degenerative changes varied from vacuolar and hydropic degenerations to cell death in rats fed low dose of 1% TiO2 (Fig 3-D). TiO2 2% resulted in more intense lesions mainly steatosis of the hepatic cells (Fig. 3-E).

Table (1): Changes in mean body weight (g) of male albino rats fed on 1% and 2%TiO2 containing rations for 65 days (Mean± S.E.).

Group	Treatment	Mean of body weight at the beginning	65 days post- administration
C	Free diet	181.00 ±5.56 [a]	275.16±2.6 [a]
T 1	TiO2 1%	179.42±5.2 [a]	237.83±1.92 [b]
T 2	TiO2 2%	182.78 ±7.04 [a]	235.66±2.18 [b]

Means in the same row having different superscript were significantly different (P< 0.05)

Table (2): Changes in epididymal sperm characters of male albino rats fed on TiO2 1% and 2% containing rations for 65 days and their serum testosterone level after 65 days. (Mean± S.E.)

Treatment	Group	Motility %	Sp.C.C/ml x125x10^5	Abnormalities %	Live %	Testosterone (ng/ml)
Free diet	C	86.66±1.66 [a]	26.33±0.88 [a]	6.4±0.61[c]	93.22±0.94[a]	1.92±0.71[a]
TiO_2 1%	T 1	82.50±2.14 [ab]	18.5±2.10 [b]	14.95±1.65 [b]	86.84±3.71[b]	0.622±0.21[b]
TiO_2 2%	T 2	72.50±3.81[b]	13.83±1.35 [b]	28.15±1.90 [a]	76.32±1.89 [b]	0.573±0.33 [b]

Means in the same row having different superscript were significantly different (P< 0.05)

Table (3): Changes in antioxidant enzymes activities in liver homogenate and serum nitric oxide level of male albino rats fed 1% and 2%TiO_2 containing rations for 65 days (Mean± S.E.)

Duration	Group	SOD (u/gm tissue)	GR (u/gm tissue)	MDA (µmol/L homogenate)	Nitrite level (umol/L)
65 days	C	30.53±2.76 [b.]	0.208±0.009 [c.]	1.46±0.09 [a]	0.4623± 0.006 [c]
	T 1	71.98 ±5.6 [a]	0.412 ±0.07 [b]	1.87±0.03 [a]	1.414± 0.04 [b]
	T 2	78.33 ±6.3 [a]	0.899 ±0.27 [a]	1.93 ±0.05 [a]	1.6405±0.04 [a]

Means in the same row having different superscript were significantly different (P< 0.05)

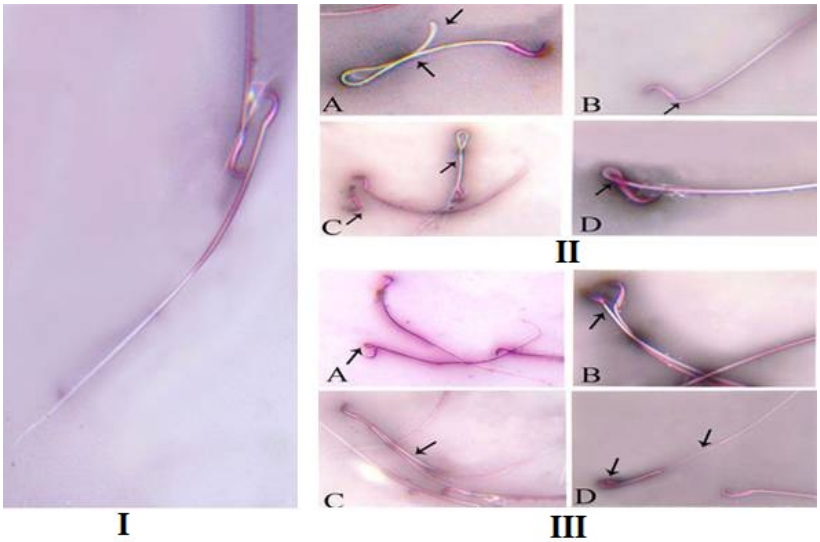

Fig. (1): Spermatozoa of male albino rats fed on No_2 free ration (control, 1-I) showing normal hock shape spermatozoa. Spermatozoa of male albino rats fed on 1% TiO_2 containing ration for 65 days showing abnormalities in the form of A) looped sperm, thickened tail. B) bent mid piece. C) Detached head and mid piece and looped sperm. D) Coiled mid piece (Fig 1-II). Spermatozoa of male albino rats fed on 2% TiO_2 containing ration for 65 days showing abnormalities in the form of A) bent mid piece. B) Abnormal hock shape. C) Double tailed sperm. D) Denuded tail, deformed hock shape (Fig 1-III).

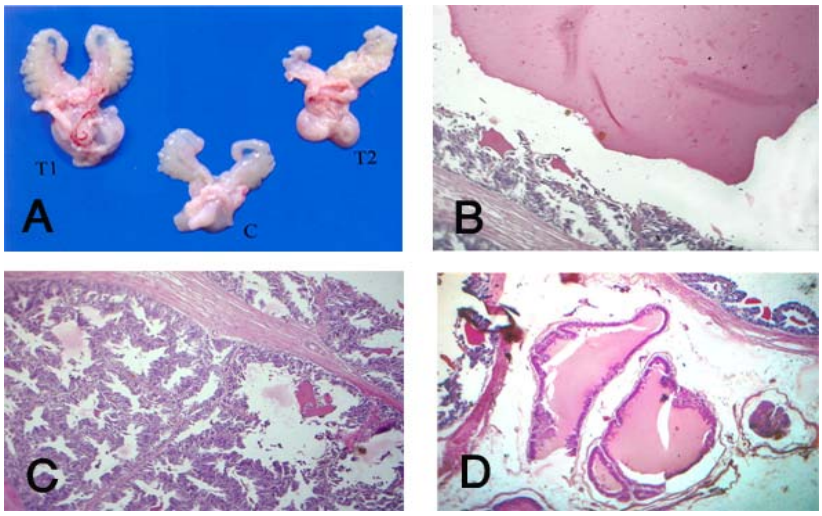

Fig. 2:

A : Gross picture of seminal vesicle of male albino rat fed TiO_2 1% showing hypertrophy T1 and $TiO_2$2% showing atrophy T2 .

B-Photomicrograph section of rat seminal vesicle control (H&E x 300).

C- -Photomicrograph section of seminal vesicle of rat fed on TiO_2 1% containing ration for 65 days showing hyperplastic glandular epithelium (H&E x 300).

D--Photomicrograph section of seminal vesicle of rat fed on TiO_2 2% containing ration for 65 days showing reduction in number and size of the epithelial lining of the tubuloalveolar gland (H&E x 300).

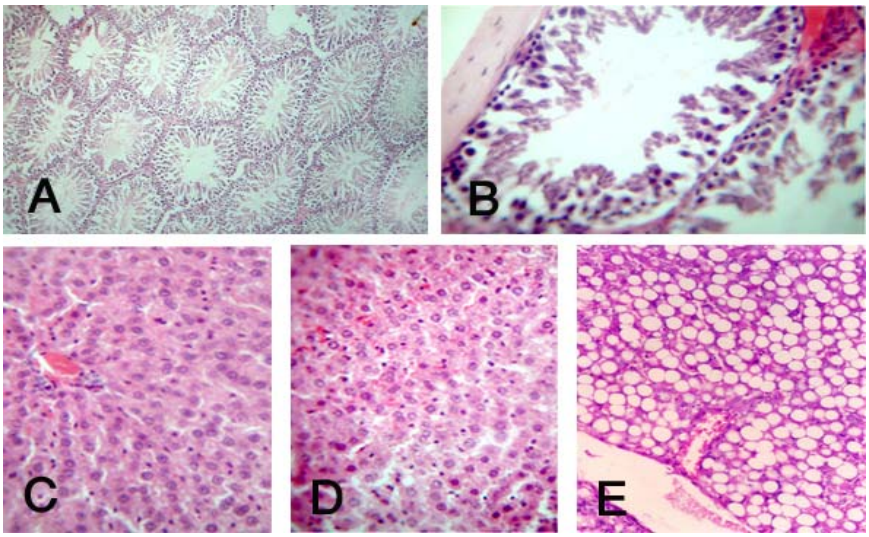

Fig. 3:

A- Photomicrograph section of rat testes (control) showing normal testicular tissue (H&E x 300).

B- Photomicrograph of section of rat testes fed on TiO_2 1% containing ration for 65 days showing mild spermatogenesis and congestion of blood vessel (H&E x 1200).

C- Photomicrograph section of rat liver (control) showing normal hepatic parenchyma (H&E x 1200).

D- Photomicrograph of section of rat liver fed on TiO_2 1% containing ration for 65 days showing vacuolar and hydropic degeneration and cell death of some hepatic cells (H&E x 1200).

E- Photomicrograph section of rat liver fed on TiO_2 2% containing ration for 65 days showing steatosis (fatty change) of hepatic cells (H&E x 1200).

4. Discussion

n the past, some food additives had been considered acceptable in the absence of adequate information. Safety data about TiO_2 is still limited and it has been needed to be evaluated repeatedly and determined its safety by Regulatory Agency in the country of use.

Regarding to the effect of TiO_2 on body weights, our results revealed that male albino rats fed on TiO_2 1% and 2% had lowered mean value of body weights if compared with that of the control group. These results are consistent with those observed by Wang et al.,(2007) who found that after acute oral administration of a single dose of TiO2 (5g/kg body weight) decreased body weight of all treated mice , Mahrousa , (2004) who recorded that oral treatment of male rats with 4mg/kg body weight TiO2 for 90 days resulted in a significant decrease in their body weight and Bermudez et al.,(2002) who exposed six week old female mice, rats, and hamsters to 10, 50, or 250 mg/m³ pigmentary TiO2 for 6 hours per day and 5 days per week for 13 weeks. TiO2 produced depression in the body weight in all species and in all groups.. The weight loss is paralleled with anorexia which was observed on the exposed animals in the present study and may be attributed to the disturbance in different metabolic systems which resulted from feeding synthetic food colorants (Abdel- Rahim et al., 1989).

Regarding the effect of TiO_2 feeding on male fertility, the present study revealed that TiO_2 1% and 2% feeding for 65 days demonstrated a significant dose dependent increase in sperm abnormalities % and significant decrease in sperm cell concentration. Sperm motility % was significantly decreased in TiO_2 2% and non significantly decreased at 1% compared with the control group. Our results were in the same context with those previously reported (Guo et al., 2009). Changes in epididymal sperm characters obtained in our result may be postulated to the generated NO following TiO_2 which plays a role in sperm motility (Herrero et al., 1997). By the same way, excessive NO production in response to a variety of stressors, possibly reducing the survival rate and motility of sperm cells (Ozokutan et al., 2000). Serum testosterone level was lowered. The high level of NO may be responsible for reduction in testosterone secretion (Adams et al., 1994), which leads to hypospermatogenesis, testicular inflammation and disturbance of GnRH secretion (Ferrini et al., 2001) and supported our histopathological evidence in the present study. Degenerated spermatogonial cell layers may be attributed to decreased testosterone synthesis and disruption of normal androgen status (Xing-

Shou, 1983) or may be due to reduced serum cholesterol level which is the precursor of all the steroid hormones (Bush,1991). TiO_2 is one of ROS generators (Sayes,et al.,2006 and Gurr et al.,2005) and confirmed by elevated antioxidant enzyme activities , SOD and GR.The present study revealed that there was a significant increase in SOD and GR enzyme activities in male rats fed on 1% and 2% TiO2.Similar results obtained after ultra-fine TiO_2 intra tracheal exposure in alveolar macrophage and peripheral RBCs of treated rats (Afaq et al., 1998) to face the high level of generated ROS mentioned .The present study showed a non significant increase MDA concentration in liver homogenate of TiO_2 treated rats. Our results are concordant with Maness et al., (1999) who mentioned that TiO_2 caused an exponential increase in the MDA production who explained an increase of lipids peroxidation due to excessive ROS generation. These results are in agreement with Gurr et al., (2005) and Olmedo et al., (2005) in human and rats bronchial epithelial cells. The pathological alterations induced by TiO_2 may be associated with the generation of ROS. Furthermore, hepatic lesions as a sequellae of accumulated TiO_2 particles which is difficultly cleared *in vivo* after oral ingestion (Wang et al., 2007).

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