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Ultrastructural Effects of Titanium Dioxide on Epithelial Cells of Small Intestine of Mice

Abdullah Melekoğlu[1], Turan Güven[2] and Hüseyin Türker[3]
[1]Kırıkkale University, Faculty of Science, Department of Biology, 71450, Yahşihan, Kırıkkale, TURKEY
[2]Gazi University, Gazi Education Faculty, Department of Biology, 06500, Teknikokullar, Ankara, TURKEY
[3]Ankara University, Science Faculty, Department of Biology, 06500, Tandogan, Ankara, TURKEY

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Abstract

In this study, the effects of different doses of Ti02 (E171) on the mucosa cells of small intestine, orally administered to Swiss albino mice (Mus musculus domesticus) have been investigated. After administration of Ti02 to mice, the changes occurred in the organelles such as nucleus, mitochondria and endoplasmic reticulum. The most prominent changes in the mucosal cells were dilation in the endoplasmic reticulum, nuclear space formation and disappeared matrix and cristae in mitochondria. Furthermore, lipid droplets were found in cytoplasm.

Keywords: Titanium dioxide, food additive coloring agent, TEM, mucosa epithelial cell, small intestine.

Introduction

The probable harmful effects of food additives have been discussed since the beginning of 20th century. Food and Agriculture Organization (FAO) and World Health Organization (WHO) have been studying guidelines and the acute, chronic and specific toxic effects of food and additives on international stage for a long time[1-3]. Today, the indispensible elements of modern food industry, the additives, present an insidious danger to human health. These additives are widely used in cosmetic and drug industry, and are also directly related to human biological systems. No matter how low amounts are taken, continuous consumption all through lifetime may cause harmful effects in long term. TiO_2 is a white pigmented additive with high opacity and coating properties. It is widely used in dye, rubber, clothing, paper, metallurgy, ceramic, and drug, cosmetic and food industries[4,5]. Three different crystal forms are present; anatase, brookite and rutile. Only anatase type is used in drug and food industries as coating material or whitener[4-6]. Scientific studies concerning additives are mainly on physiological and clinical basis. Most of them are focused on toxic dose, carcinogenicity and allergy[6-17]. It is clear that, further investigation from molecular to cellular level are needed in order to clarify the consequences of additives entered with ingestion, inhalation or dermal route. Effects of titanium on ultrastructural level are yet to be clarified. In this study, the ultrastructural effects of orally administered TiO_2 on small intestine mucosa epithelia cells, on electron microscopic level, were investigated.

Material and Methods

Twenty seven mice, weighing between 18-22 g were used in comply with WHO laboratory animal standards. All mice were supplied from Refik Saydam Hıfzıssıhha Institute of Ankara (Turkey) and the study was accomplished in Biology Department Laboratory of Ankara University Science Faculty, where a circadian period of 12 hours was maintained. Three mice were separated as control group and the remaining 24 animals were administered different concentrations of anatase form of TiO_2, orally for varying time periods. TiO_2 was not given to control group. All experiments were repeated for three times. Dosage and exposure times of the study groups were enlisted in table 1. Anesthesia of the animals was done with 0.01 mg ketamine injection intramuscularly. Specimens from the small intestine of the mice were obtained at the beginning of the study in control group and after appropriate exposure times in experimental groups. For electron microscopy, tissues of 1 mm3 were fixed in glutaraldehyde (3%) and phosphate buffered saline (pH 7.2) at 4 degrees centigrade for 3 hours, and post-fixed with 1% osmium tetroxide for 1 hour. Osmium tetroxide was washed away with the same buffered. Ethyl alcohol was used for dehydration and specimens were embedded in Araldite CY-212. Thin sections were double stained with saturated uranyl acetate (20 min.) and lead citrate (10 min). Jeol JEM 100 CX-II electron microscope was used for examination of the specimens.

Results and Discussion

Group I: Control Group: Primary function of the small intestine is absorption. As in other organs, form of the small intestine is closely related with structure and function. Small bowel wall may grossly be divided into four distinct layers; mucosa, sub mucosa, external muscle and serosa. In order to accomplish absorption, a wide luminal contact surface is mandatory; macroscopic plica circulares to tiny microvilli serve this purpose. Goblet cells, paneth cells, entero chromaphin cells, undifferentiated cells, and differentiated villous epithelium (enterocytes and absorptive cells) can be defined histological.

Table-1
Experimental groups, dosage and exposure times

Study groups	Number of mice in experimental groups	Daily dosage was given (g/mouse/n)	Exposure time (days)	Total dosage (g)
Group I (Control group)	3	not given	20	0,0
Group II (Low dose administration of TiO2)	3	0.003 g	5	0.015
Group III (Moderate dose of TiO_2 administration)	3	0.03 g	16	0.48
Group IV (High dose of TiO_2 administration)	3	0.3 g	16	4.8
Group IV (High dose of TiO_2 administration)	3	3.0 g	16	48
Group IV (High dose of TiO_2 administration)	3	3.0 g	17	51
Group IV (Excessively high dose of TiO_2 administration)	3	3.0 g	18	54
Group IV (Excessively high dose of TiO_2 administration)	3	3.0 g	19	57
Group IV (High dose and excessively high dose of TiO_2 administration)	3	3.0 g	20	60

Absorptive cells are the most common and most active cells of the villous surface. These are simple cylindrical cells with brushy appearance stretching out intraluminally. Well organized microvilli can be detected microscopically, on this brushy surface. Absorptive cells with microvilli accomplish the absorption process. The epithelial structure is a complex unit, regulating the passage to lamina propria. Integrity of this tissue requires proliferation, maturation and metabolic activity of the cells. Failure in one of these steps results small intestine malfunction and malabsorption.

Absorptive cells are long and cylindrical cells. Basally located nucleus is in harmony with the shape of the cell. Microvilli are located on the luminal side of the cell. Apical cytoplasm contains numerous mitochondria and endoplasmic reticulum. Cellular tight junction zones are observed. These zones are most distinct in adjacent lateral membranes of the luminal side of the neighboring cells (figure 1, figure 2).

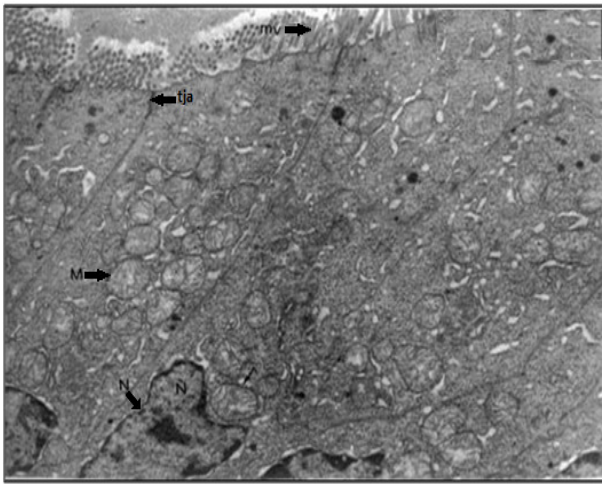

Figure-1
Normal absorptive small intestine cells of the group I (Control group). Tight junction area (tja), mitochondria (M), microvilli (mv) and nucleus (N). x9.600

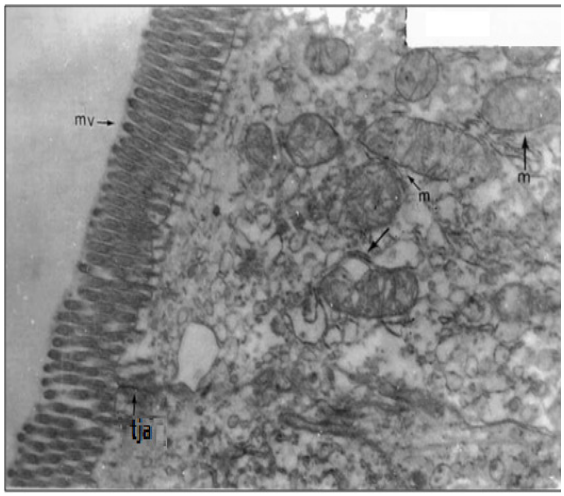

Figure-2
Microvilli and apically located mitochondria in control group. Tight junction area (tja), mitochondria (M) and microvilli (mv). x20.000

Group II: Low dose administration of TiO_2: After 5 days of 0.003 g/day ingestion of TiO_2, electron micrograph of the absorptive cells revealed nuclear and mitochondrial elongation, early stages of cristae and matrix loss in mitochondria. On the other hand microvilli structure was preserved in this group (figure 3, figure 4).

Group III: Moderate dose administration of TiO_2: After 16 days of 0.03 g/day ingestion, Ultrastructure of the absorptive cells showed significant changes. Cristae loss and space formation in apical mitochondria, alterations in nuclear and nucleolar structure, dense helesonic granule formation in nucleus, nuclear heterochromatin and euchromatin loss, electron lucency of the karyoplasms, distortion in microvilli, Cytoplasmic spaces and dissolution with appearance of lipid droplets of varying sizes. Most striking histopathological finding in group III was cytoplasmic lipid droplets (figure 5, figure 6, figure 7 and figure 8).

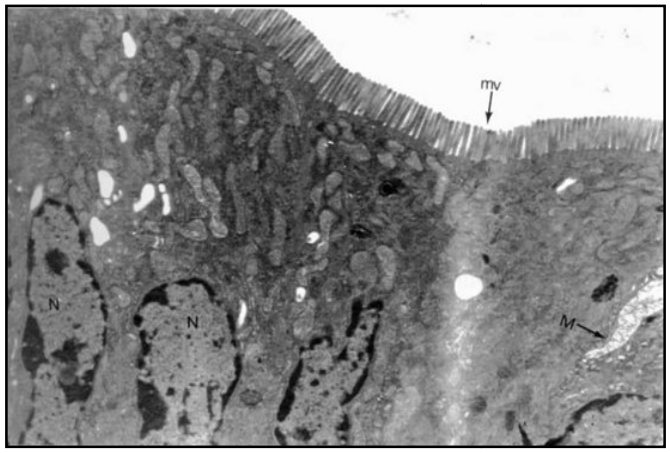

Figure-3
Elongated mitochondria in group II. An elongated mitochondrion on the right side. Mitochondria (M), microvilli (mv) and nucleus (N). x9.600

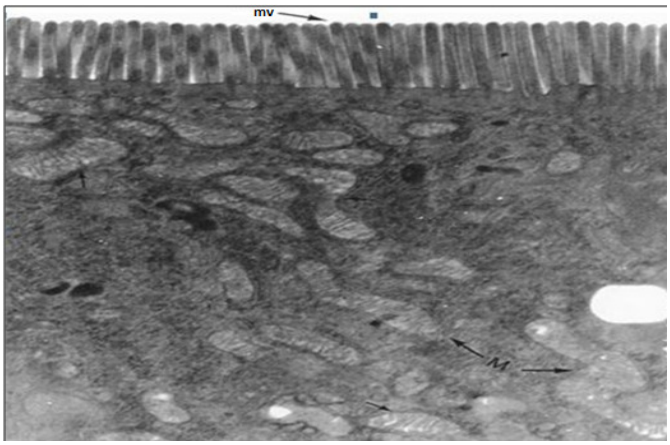

Figure-4
Preserved microvilli structure (mv), with loss of cristae and mitochondrial elongation (M) in group II. X 20.000

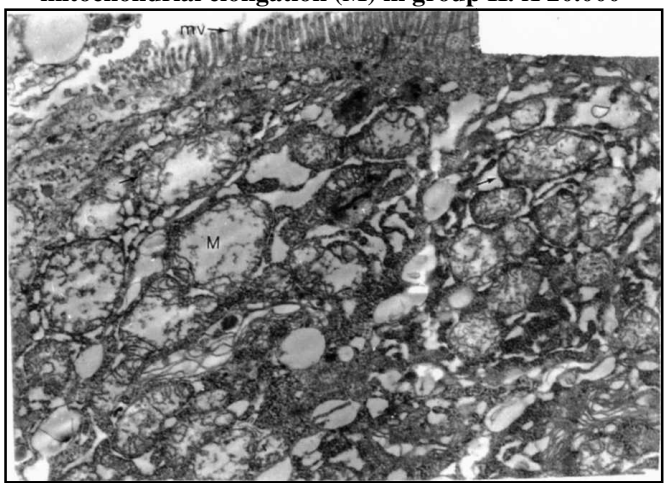

Figure-5
Mitochondrial cristae loss (M) and space formation due to matrix dissolution in group III. Alterations in microvilli (mv) can be observed. x14.400

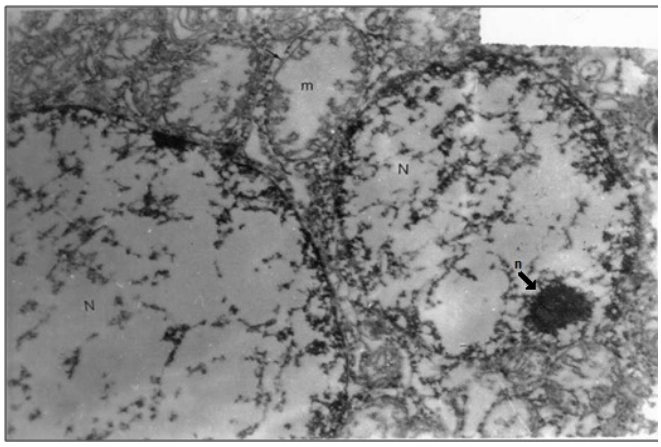

Figure-6
Nuclear matrix dissolution and formation of large space in small intestine epithelial cells of mice in group III. Mitochondrial vacuolization (m) increased and significant changes happened in nucleolus (n). x20.000

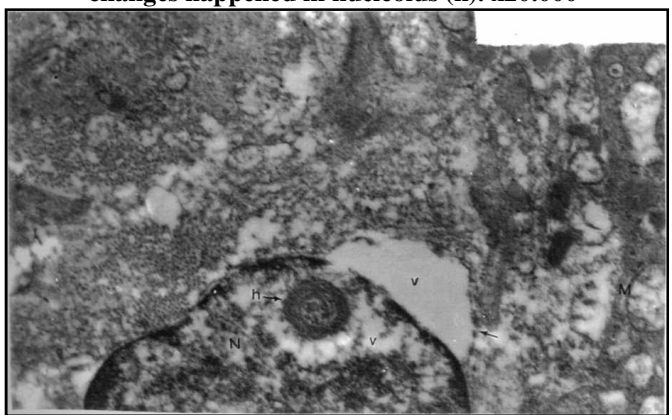

Figure-7
Electron dense helesonic granule (H) in nucleus (N) and abnormal space formation in perinuclear zone neighboring granule (v) in group III. Mitochondrial matrix (M) loss also was occured. x28.000

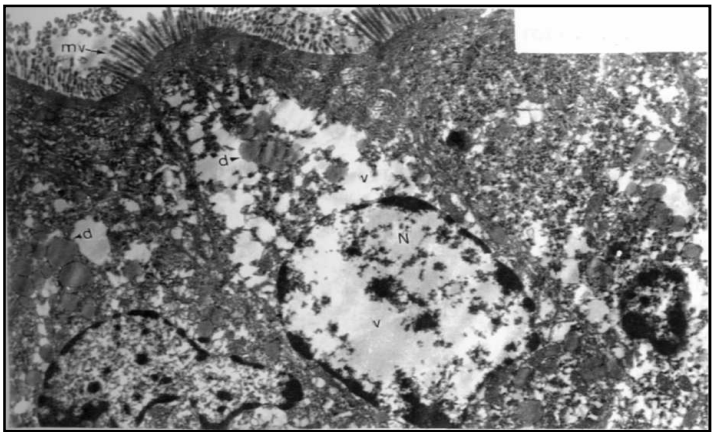

Figure-8
Nuclear (N) and cytoplasmic space (v), distortion in microvilli (mv) and numerous round cytoplasmic lipid droplets (d) in group III. x9.600

Group IV: High dose and excessively high dose administration of TiO_2 : Conspicuous changes in nucleus, mitochondria and endoplasmic reticulum of the epithelial cells were noted group IV (figure 9-14). Abnormal dilation between nuclear membranes and distortion of mitochondrial matrix were happened after 16 days of high dose of TiO_2 ingestion (figure 9); perinuclear space and endoplasmic reticulum enlargement, nuclear and mitochondrial distortion was occurred after 17 days of high dose of TiO_2 ingestion (figure 10, figure 11); cytoplasmic and nucleoplasmic changes, dilation between nuclear membranes, electron dense granules, endoplasmic reticulum dilatation and vesicles in dilated regions, mitochondrial dilatation, matrix and crystal dissolution, lipid droplets and numerous vesicles were seen after 18 days of high dose of TiO_2 ingestion (figure 13-15); swelling of endoplasmic reticulum with electron dense material accumulation were occured after 19 days of high dose of TiO_2 ingestion (figure 16); dilatation of ER, distortion of nucleus, nuclear membrane and double layered mitochondrial membrane was happened after 20 days of high dose of TiO_2 ingestion (figure 17).

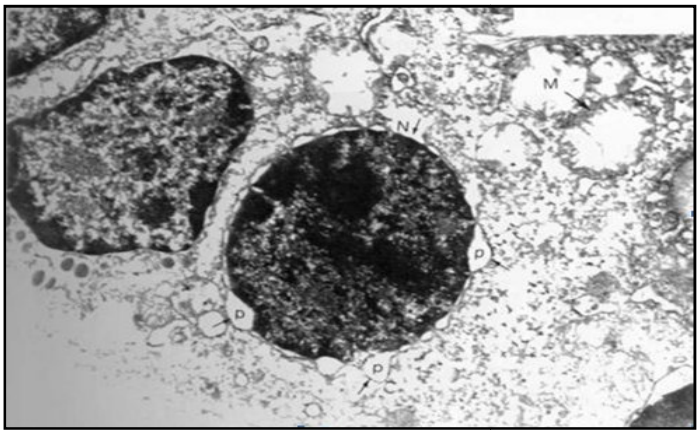

Figure-9
Mitochondrial matrix distortion and separation between nucleolemmal membranes of the mice small intestine epithelial cell in group IV. Nucleus (N), perinuclear dilation (p) and distorted mitochondria (M). x14.400

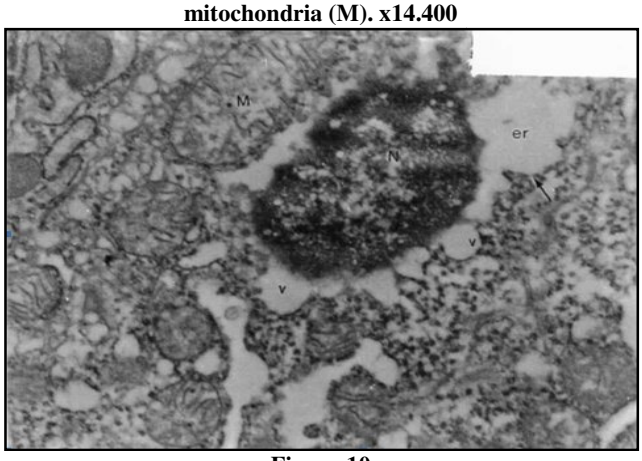

Figure-10
After 17 days of 3 g/day of TiO_2 ingestion, perinuclear space and dilatation of endoplasmic reticulum (er) and mitochondria (M) in epithelial cells of mice in group IV. x 28.000

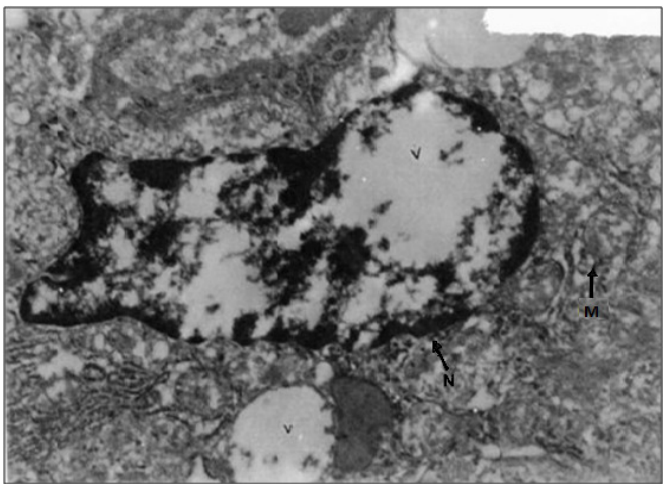

Figure-11
Nuclear (N) and mitochondrial distortion (m) and space formation (v) in epithelial cells of mice in group IV. x20.000

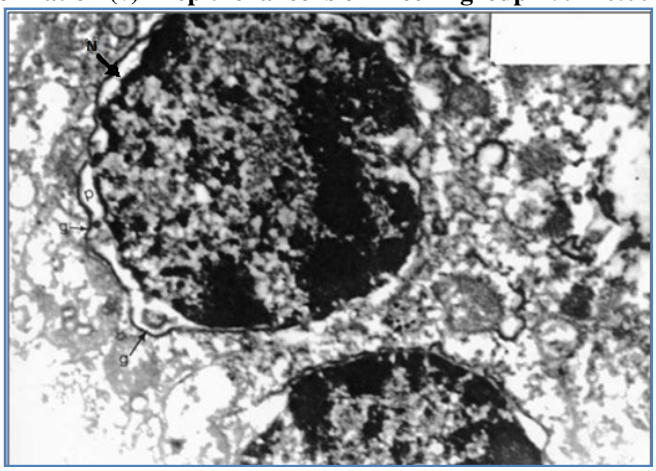

Figure-12
Perinuclear dilation, cytoplasmic distortion, electron dense area in the nucleus (N), granules (g) in perinuclear space. x20.000

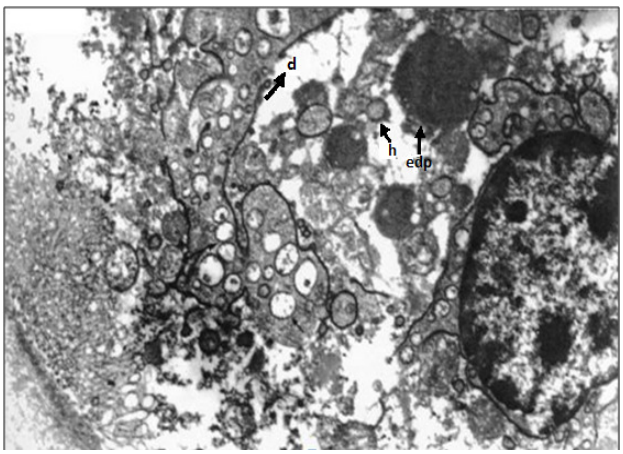

Figure-13
Dilatation of endoplasmic reticulum (d), vesicles in dilated zones (h) and electron dense particles in endoplasmic reticulum (edp) in group IV. Cytoplasmic vacuoles and electron dense materials are the significant pathologic findings. x14.500

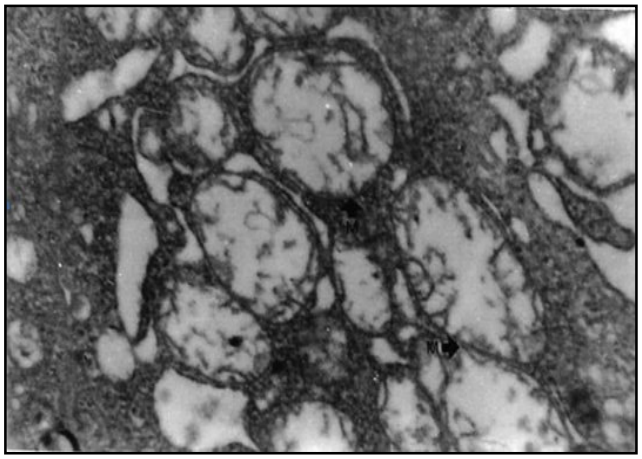

Figure-14
Mitochondrial cristae loss (m) and matrix dissolution in group IV. x28.000

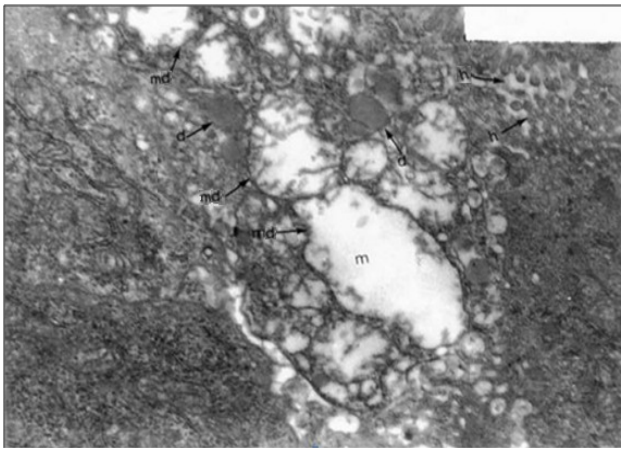

Figure-15
Excessive swelling of mitochondria (m) and matrix dissolution (md), cytoplasmic lipid droplets (d), and numerous vesicle formations (h) in group IV. x20.000.

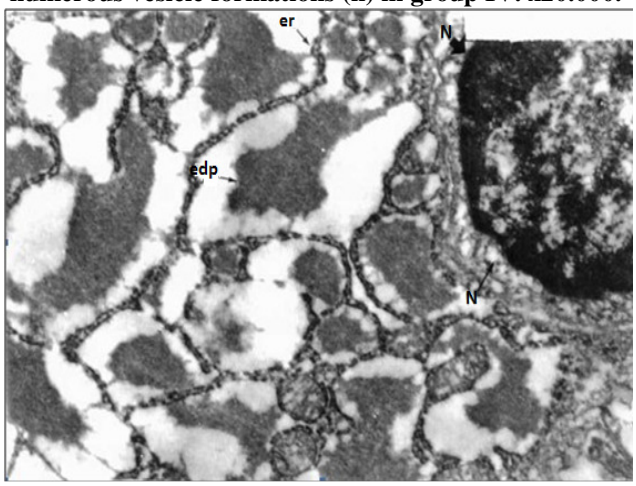

Figure-16
Excessive swelling of endoplasmic reticulum (er) and electron dense materials (edp) in group IV. x20.000

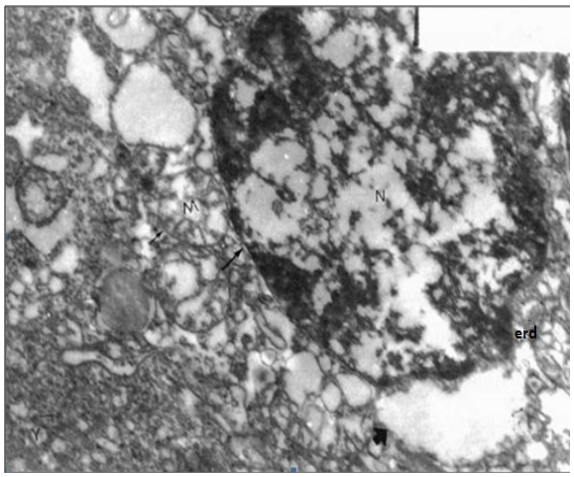

Figure-17
Endoplasmic reticulum dilatation (erd), distortion in mitochondrial membrane (M) and necrosis of nucleus (N) (thick arrow) in group IV. x20.000

Discussion: Although titanium is widely used as a food additive there are not many experimental studies concerning its effects. Specific studies about the effects of titanium dioxide on biologic system are not present or very few in literature, to our knowledge. Recent data proposes that titanium is not toxic, so it is safe to use[3,12,18-20]. As a result, with high biocompatibility and osteointegration, titanium is the most popular material in dentistry, orthopedic and reconstructive surgery.

As, TiO_2 is an inert compound and dissoluble in water, it seems reasonable to use it as a food additive. Although short term and low dose usage still seems to be safe, findings reported with long term and high dose usage interrogated their safety. Sensitivity of methods is also important in an experimental study. As an example, 50 mice and 50 rats were fed with 25.000- 50.000 pp TiO_2 for 103 weeks and no significant weight change could be documented at the end of the study[9].

TiO_2 is accepted to be non carcinogenic but ingestion of titanium by some specific cells may trigger tissue damage. Specific and reliable methods were used for this purpose. After orally administration (gavages) of 12.5 mg/kg dose of 500 nm sized TiO_2 (rutile) particles for 10 days, light microscopic and SEM examination revealed that particles are present in small intestine, colon, mesentery, peritoneum, lung, liver and spleen whereas no particles could be detected in cardiac and renal tissue[2,12,15,21]. On the other hand, TiO_2 has shown to infiltrate all organs and lymphoid tissue, in histochemical analysis[12].

In a study concerning the histopathological and ultrastructural effects of TiO_2 particles in canine lungs, detrimental effects were documented. Following intra- tracheal exposure to TiO_2 dusts for 9-15 months of 16 dogs, SEM and energy dispersive X-ray (EDX) examinations documented the presence of TiO_2 in bronchi and alveoli with mild alveolar fibrosis[18,20].

It was reported that 3% of the ingested TiO_2 was absorbed and the rest was excreted[19]. TiO_2 was reported to cause epidermal and corneal damage in rabbits, whereas no toxic effects with long term exposure could be documented in rodents[19,22].

Probable detrimental effects of orally taken inert particles, during and after the absorption process through stomach lumen have been suggested[12]. Lung damage with TiO_2 inhalation was shown in mice, rats, dogs and human[10,12,19,23]. High concentration exposure via inhalation was shown to result in lung tumor and keratin cyst formation[21]. Toxic effects of titanium nanoparticles on mice brain, liver, lung, bone marrow, nervous system, retina, gastric antrum and colon cells was also reported[12, 16,17,24].

Studies concerning effects of TiO_2 inhalation are limited. Examination of worker lungs exposed to TiO_2 dusts revealed contradictory results. Some workers had no findings but others showed pathological changes[9]. TiO_2 dust exposure has been reported cause fibrotic and hyperplasic changes in lungs, skin and lymphoid tissue. These tissues were shown to have numerous black particle containing granules- which were demonstrated to be TiO_2 particles by electron microscope and EDX[12,19]. On the contrary, no harmful effect on human health could be validated in all these studies[19,26].

Although metabolism and biologic behavior of TiO_2 is not precisely known, in the light of the data derived from earlier studies, TiO_2 is thought to be a low potent toxic agent[19].

It is reasonable to anticipate cellular changes after TiO_2 administration. This is due to first contact with small intestine epithelium cells before disseminating systematically. In the recent studies, traces of TiO_2 were shown also in blood, lung, liver, brain, cardiac, stomach wall, bone marrow and spleen tissue[2,10,12,14,16,17,23,25].

Cellular pathologic effects of TiO_2 could not be documented in none of these studies as all of them were biochemical, light microscopic or TEM studies. In these experimental, different doses of TiO_2 was administered orally for varying time periods and electron microscopic assessments were carried out on the basis of control group to reveal the pathologic changes in small intestine epithelium cells. Correlated with the exposure time and dose, most significant changes were observed in mitochondria, nucleus and endoplasmic reticulum. Cytoplasmic spaces and lipid droplets were also remarkable. As well as that, loss of integrity of cytoplasm of small intestine epithelium, cytoplasmic spaces and vacuole formation was noted.

We cannot able to reach any other study on ultrastructural levels; comparison of the results could not be accomplished. Further studies are required to clarify the effects of TiO_2 on cellular level.

Conclusion

In this experiment was analyzed the TiO2 nanoparticles effect, doped or not with a metal, at the intestinel level in Mus musculus domesticus, and their interaction with the cellular organelles. The TiO2 nanoparticles, induced a drastic effect at the epitheliel cell level, affecting the nucleus and some organelles ultrastructure of organelles (mitochondria especially), as well as the lipid metabolism.

References

1. FAO/WHO, Evaluation of certain food additives and contaminants. Report of the joint FAO/WHO expert committee on food additives, *WHO Technical Report Series*, 806 **(1991)**

2. Gurr J.R., Wang A.S., Chen C.H. and Jan K.Y., Ultrafine titanium dioxide particles in the absence of photo activation can induce oxidative damage to human bronchial epithelial cells, *Toxicol*., **213(1-2)**, 66-73 **(2005)**

3. Hallagan J. B., Ailen D.C and Borzelleca J.F., The Safety and Regulatory Status of Food, Drug and Cosmetics Color Additives Exempt from Certification, *Food and Chemical Toxicol*., **33(6)**, 515-528 **(1995)**

4. Sahrul S., Rita P., Marina I.H., Pepen A., Khairurrijal and Mikrajuddin A., Efficiency Improvement in Tio2-Particle Based Solar Cells After Deposition of Metal in Spaces Between Particles, *Int. J. Basic & Applied Sc*., **11**, 15-28 (**2011)**

5. JECFA, Joint FAO/WHO Expert Committee on Food Additives. Titanium dioxide in combined compendium of Food Additive Specifications, *FAO*, Roma., **3 (2006)**

6. Mano S.S., Kanehira K., Sonezaki S. and Taniguchi A., Effect of Polyethylene Glycol Modification of TiO_2 Nanoparticles on Cytotoxicity and Gene Expressions in Human Cell Lines, *Int. J. Mol. Sci.,* **13(3)**, 3703-3717 **(2012)**

7. Sungkaworn T., Triampo W., Nalakarn P., Triampo D., Tang I. M., Lenbury Y. and Picha P., The Effects of TiO2 Nanoparticles on Tumor Cell Colonies: Fractal Dimension and Morphological Properties, *Int. J. Biol. Life Sci.*, **2(1)**, 67-74 (**2006)**

8. AL-Shinnawy M., Physiological effect of a food additive on some hematological and biochemical parameters of male albino rats, *Egypt. Acad. J. Biol. Sci.,* **2(1),** 143-151 **(2009)**

9. Boffetta P., Bioassay of $Ti0_2$ for possible carcinogenicity (CAS No: 13463 - 67 - 7), *Int. J. Pharm.* **107(3)**, 117-128 (1995)

10. Afaq F., Abidi P., Matin R. and Rahman Q., Cytotoxicity, pro-oxidant effects and antioxidant depletion in rat lung alveolar macrophages exposed to ultrafine titanium dioxide, *J. Appl. Toxicol.,* **18(5)**, 307–312 **(1998)**

11. Chen, E., Ruvalcaba, M., Araujo, L., Chapman, R. and Chin, W.C., Ultrafine titanium dioxide nanoparticles

induce cell death in human bronchial epithelial cells. *J. Exp. Nanosci. 3*, 171–183 **(2008)**

12. Sycheva L.P., Zhurkov V.S., Iurchenko W., Daugel-Dauge N.O., Kovalenko M.A., Krivtsova E.K. and Durnev A.D., Investigation of genotoxic and cytotoxic effects of micro and nanosized titanium dioxide in six organs of mice in vivo, *Mut. Res*., **726(1)**, 8-14 **(2011)**

13. Huang K,. Chen L., Liao M. and Xiong J., The Photocatalytic Inactivation Effect of Fe-Doped TiO2 Nanocomposites on Leukemic HL60 Cells-Based Photodynamic Therapy*, Int. J. Phot.,* 1-8) **(2012)**

14. Yang H., Qin X., Tian A. Zhang D., Xue X. and Wu A., Nano Size Effects of TiO2 Nanotube Array on the Glioma Cells Behavior, *Int. J. Mol. Sci.,* **14**, 244-254 **(2013)**

15. Nabela, I., EL- Sharkawy* , Salah, M. Hamza and Ehsan, H., Abou-Zeid, Toxic Impact of Titanium Dioxide (TiO2) In Male Albino Rats with Special Reference to its Effect on Reproductive System, *J. Am. Sci*., **6(11)**, 865-872 **(2010)**

16. Iavicou I., Leso V., Fontana L., and Bergamaschi A., Toxicological effects of Titanium dioxide nanoparticles: a review of in vitro mammalian studies, *Eur. Rev. Med. Pharm. Sci*., **15**, 481-508 (**2011**)

17. Iavicou I, Leso V. and Bergamaschi A., Tocxicological effects of Titanium Dioxide Nanoparticles: A Review of In Vivo Studies, *J. Nanomaarials*, 36 (**2012**)

18. Zeng L., Zheng Z. and Zhang S., Pathogenic effects of $Ti0_2$ dust on the lung of dogs, *Med. Sci*., **20(1)**, 88-91 **(1989)**

19. Cesar A.M., Florabel G., Mullick M.D., Kamal G., Ishak M.D., Frank B., Johnson M.D., William B. and Hummer B.S., Identification of Titanium in Human Tissues: Probable Role in Pathologic Processes, *Hum. Pathol.*, **22(5)**, 450-454 **(1991)**

20. Donaldson K., Brown M.G., Brown D.M., Robertson D.M., Slight J., Cowie H., Jones A.D., Bolton R.E. and Davis J.M.G., Contrasting Bronchoalveolar Leukocyte Responses in Rats Inhaling Coal Mine Duts, Quartz, or Titanium Dioxide: Effects of Coal Rank, Airborne Mass Concentration and Cessation of Exposure, *Envir. Res.*, **52**, 62-76 **(1990)**

21. Rosenberg K.W., Gratz H.H. and Sailer F., Should titanium manipulates be removed after bone healing is complete? *Int. J. Oral Max. Surg*., **22**, 185-188 **(1983)**

22. Nicola I., Stefanelli G., Valletrisco M. and Amato P., Effects of a diet containing titanium dioxide on albino rats, *Industrie-Alimentari*., **16(1)**, 88-92 (**1977**)

23. Praful U.J., Mc Carthy D.E. and Alexander T., Titanium dioxide (rutile) particle uptake from the rat Gl tract and translocation to systemic organs after oral administration, *Int. J. Pharm*., **105(2)**, 157-168 **(1994)**

24. Driscoll K.E., Lindenschmidt R.C., Maurer J.K., Perkins L., Perkins M. and Higgins J., Pulmonary response to inhaled silica or titanium dioxide, *Toxicol. and App. Pharm.,* 111(2), 201–210 **(1991)**

25. McMillan H., Jones A.D., Vincent J.H., Johnston A.M., Douglas A.N. and Cowie H., Accumulation of Mixed Mineral Dust in the Lungs of Rats During Chronic Inhalation Exposure, *Envir. Res.*, **48**, 218-237 **(1988)**

26. Schilling K., Bradford B., Castelli D., Dufour E., Nash J.F., Pape W., Schulte S., Tooley I., van den Bosch J. and Schellauf F., Human safety review of "nano" titanium dioxide and zinc oxide, *Photochem. Photobiol. Sci.*, **9(4)**, 495-509 **(2010)**