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Impacts of Excessive Alpha-Tocopherol and Retinoic Acid on Cell Proliferation During Mouse and Placenta Development via CDKN1B Activation

Impactos del Exceso de Alfa-Tocoferol y Ácido Retinoico en la Proliferación Celular Durante el Desarrollo de Ratones y Placenta Mediante la Activación de CDKN1B

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SUMMARY: Vitamins such as vitamin A and vitamin E are used in many therapeutics and cosmetics. They also play a vital role in fetal development during pregnancy. Therefore, this study aims to explore the negative effects of high doses of retinoic acid and alpha-tocopherol during embryo development and placentation. Fifteen pregnant mice were divided into three groups (n=5/group); (1) the control (received dimethyl sulfoxide (DMSO) and diluted with sunflower oil), (2) the Alpha-Toc treated (received 50 mg/kg/day dose), and (3) RA-treated (received 10 mg/kg/day dose). Intraperitoneal injections were given daily with a volume of 0.1 ml on gestational days 10.5, 11.5, and 12.5. The animals were euthanized by cervical dislocation on day 13.5 of gestation. Histological and immunolocalization of CDKN1B were performed. Histopathologically, the intestine, liver, and placenta were unaffected by Alpha-Toc; in contrast, RA caused severe malformations during histogenesis of the studied organs compared to a control group. Immunohistochemically, our findings revealed the upregulation of CDKN1B immunostaining by both Alpha-Toc and RA in varying proportions. We found that alpha-tocopherol and retinoic acid upregulated CDKN1B expression, which affected the histogenesis of the intestine, liver, and placenta, but via retinoic acid, not via alpha-tocopherol.

KEY WORDS: Alpha-Toc; Histogenesis; Intestine; Liver; Placenta development; RA.

List of Abbreviations

Abbreviations	Name
Alpha-Toc	α-tocopherol
CDKN1B (CDKip27)	Cyclin-dependent kinase inhibitor 1B
CYP26A1/Cyp26a1	cytochrome P26 A1
DAB	3,3'-diaminobenzidene
EGFR	epidermal growth factor receptor
Gam-Toc	gamma-tocopherol
GD	gestational day
LRAT/Lrat	lecithin-retinol acyltransferase
PCD	programmed cell death
PKC	protein kinase C
RA	Retinoic acid
RALDH	retinaldehyde dehydrogenases
RARB	retinoic acid receptor beta
RDH/Rdh	retinol dehydrogenases
ROS	reactive oxygen species
VE	Vitamin E

INTRODUCTION

The research on vitamin E (VE), including α, β, γ, δ tocopherols and α, β, γ, δ tocotrienols for disease prevention and therapy, has primarily concentrated on a-tocopherol (Alpha-Toc), recognized as the most potent antioxidant among VE forms (Jang & Kim, 2024). VE is an essential micronutrient in the human body. VE maintains various body functions. It is very crucial in maternal health and child development (Bastani *et al*., 2011). VE is an important fat-soluble micronutrient for higher mammals and functions as an antioxidant for lipids (Takada & Suzuki, 2010). VE supplementation may help reduce the risk of pregnancy complications involving oxidative stress. There is a need to evaluate the efficacy and safety of VE supplementation in pregnancy (Rumbold *et al*., 2015). A lack of VE can lead to female infertility, miscarriage, premature delivery, eclampsia,

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fetal intrauterine growth restriction, and other diseases associated with pregnancy (Hubalek *et al*., 2014; Wahid *et al*., 2014). Alpha-Toc has strong redox activity (Neuzil *et al*., 1997), has been shown to inhibit protein kinase C (PKC) (Azzi & Stocker, 2000). Furthermore, it has been reported to inhibit apoptosis induced by various stimuli, probably due to its radical scavenging capacity, as many models of apoptosis (programmed cell death, PCD) involve the generation of reactive oxygen species (ROS) (Haendeler *et al*., 1996).

Retinoic acid (RA), a physiologically active metabolite of vitamin A (retinol), plays a vital role in reproduction (Clagett-Dame & Knutson, 2011). RA bioavailability is regulated by multiple factors (Bushue & Wan, 2010), such as the storage of vitamin A as retinyl esters by lecithin-retinol acyltransferase (LRAT/Lrat), RA synthesis by retinol dehydrogenases (RDH/Rdh), and retinaldehyde dehydrogenases (RALDH), and RA inactivation by cytochrome P26 A1 (CYP26A1/Cyp26a1), as well as binding proteins and retinol carrier transthyretin (Diao *et al*., 2010), *etc.* RA signaling involves RA bioavailability and RA binding to retinoic acid receptors and retinoid X receptors (Honglu *et al*., 2024). RA is tightly regulated during embryogenesis as a signaling molecule essential for forming organs such as the spinal cord, body axis, eyes, limbs, heart, and kidneys (Yang *et al*., 2021; Said *et al*., 2022). Furthermore, RA has been implicated in extraembryonic tissue development and germ cell differentiation (Cheng *et al*., 2017; Lemke *et al*., 2024). RA upregulates cyclin-dependent kinase inhibitor 1B (CDKN1B), which halts the cell cycle necessary for differentiation, emphasizing the potential adverse impact of RA on brain development (Said *et al*., 2024).

CDKN1B/p27^{Kip1} (hereafter p27) is an intrinsically disordered protein belonging to the CIP/Kip family of cyclin-dependent kinase modulators (Bencivenga *et al*., 2021). The protein represents a critical regulator of the cellular responses to various stimuli and environmental factors, including signals that inhibit proliferation or promote growth, differentiation, and DNA damage (Movassagh & Philpott, 2008; Choi *et al*., 2015; Russo *et al*., 2020). Through a Kinase Inhibitory Domain located in its N-terminal region, p27 regulates the activity of nearly all CDK/Cyclin complexes, thereby playing a crucial role in controlling the progression of the cell cycle (Bencivenga *et al*., 2021). When p27 is localized in the nucleus, it binds to Cyclin E/CDK2, blocking the kinase activity and preventing the transition from the G1 to the S phase. In turn, this can lead to G1 arrest and, in some cases, cause cells to enter a resting state (G0) (Bencivenga *et al*., 2025). Here we present the investigation of comparative effects on the cell cycle during mouse intestine, liver, and placenta development by administration of alpha-tocopherol (Alpha-Toc) and retinoic acid (RA) for modulating CDKN1B immunoexpression.

MATERIAL AND METHOD

Ethical approval. The experiment was conducted on albino mice (Swiss strain), following the standard operating procedures approved by the Faculty of Science's Ethics Committee for Animal Experimentation at Al-Azhar University in Egypt (Permit Numbers: AZHAR9/2023 & AZHAR2/2024). All animal experiments are conducted according to the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines and carried out following the National Institutes of Health guide for the care and use of laboratory animals.

Experimental design. Adult fertile male (n=8) and virgin female (n=15) albino mice (Swiss strain) were obtained from the animal house of the Experimental Animal Unit, National Cancer Institute, Cairo University. The environment in which Swiss mice were housed throughout the experiment was kept under rigorous control at the animal house, with the availability of adequate food and water. Adult virgin females (25-28 g), were mated with fertile males at an age of 3–4 months and tested for the presence of a vaginal plug. The morning of the vaginal plug was designated as day 0.5 of pregnancy. The pregnant mice were divided into three groups of 5 animals each; (1) the control group which received dimethyl sulfoxide (DMSO) and diluted with sunflower oil with a volume of 0.1 ml, (2) the Alpha-Toc treated group which received 50 mg/kg dose, and (3) RA-treated group receiving a dose of 10 mg/kg. The Alpha-Toc treated group received a daily oral administration of dosage diluted with sunflower oil with a volume of 0.1 ml, and the RA-treated group received a daily intraperitoneal injection (IP) of dosages dissolved in DMSO diluted with sunflower oil with a volume of 0.1 ml on gestational days 10.5, 11.5, and 12.5. The animals were physically euthanized by cervical dislocation on day 13.5 of gestation. Both uterine horns were removed. The embryos were isolated for histological and immunohistochemical techniques.

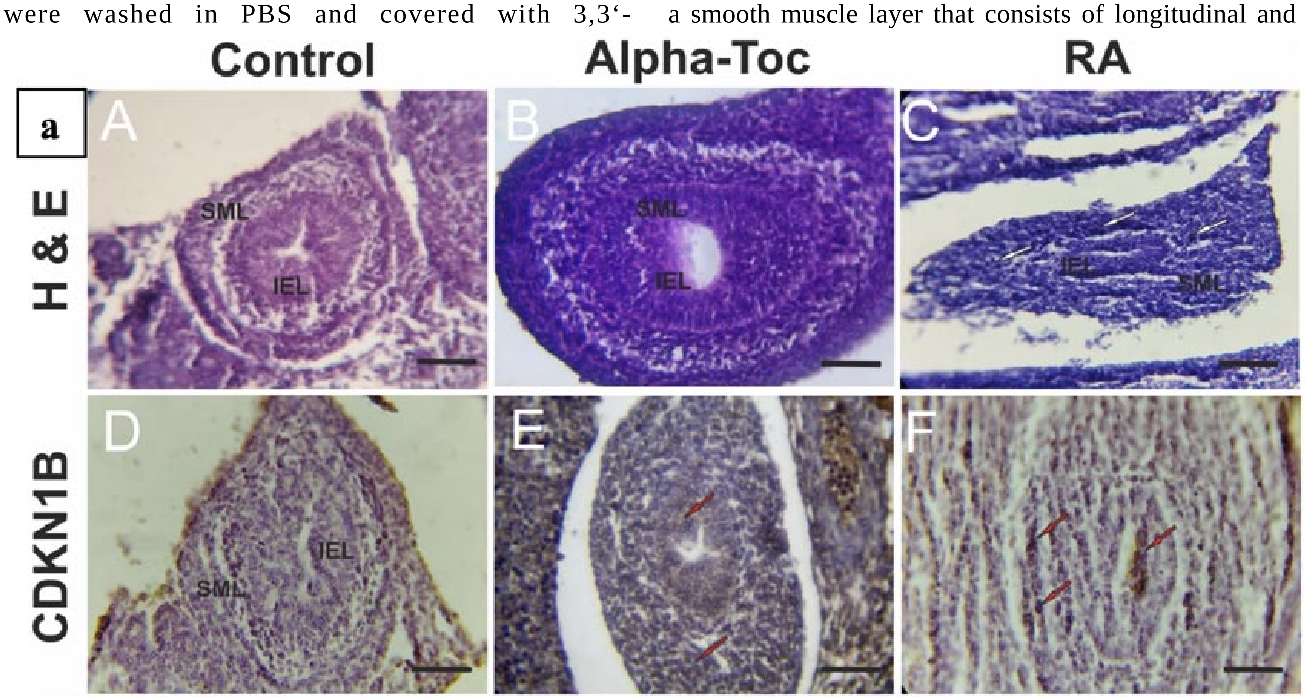
Tissue preparation for histology. Carnoy's fixative for embryo fixation is about 2 hours. Then, embryos were dehydrated using an ascending ethanol series (70 %, 90 %, and 100 %) with 90-minute washes at each concentration, and they were subsequently processed for paraffin wax embedding. Embryos were embedded for transverse sectioning at each time point. Serial 7-µm sections spanning the entire embryos were placed on clear slides and routinely stained with hematoxylin and eosin (H&E) for histological analysis according to the previous protocol (Seleem *et al*., 2018). The sections were examined and photographed using a Toupcam camera.

Immunostaining. For the immunohistochemical method, embryo tissues were fixed in Carnoy's fixative and then dehydrated. Paraffin sections, 7 µm thick, of tissues were mounted on coated slides and then dewaxed in xylene. The slides were hydrated in 100 %, 90 %, 70 %, and 50 % ethanol, then immersed in water. Endogenous peroxidase was blocked by incubation with 3 % H_2O_2 in phosphate buffer solution (PBS) for 10 min. The slides were washed in PBS and then incubated with a 10 % protein-blocking solution (EconoTek Superblock) to block the nonspecific binding sites of the antibodies. Subsequently, the slides were incubated with the primary antibody, a polyclonal CDKN1B ($p27^{kip1}$) antibody (Anti-CDKN1B Rabbit polyclonal IgG, Spring Bioscience, Canada), diluted 1:200 in PBS. After extensive washing in PBS, slides were incubated for 30 min with a biotin-conjugated secondary antibody (EconoTek Biotinylated Anti-Polyvalent). To visualize bound antibodies, sections were washed in PBS and covered with 3,3'- a Toupcam camera was used to inspect and photograph the individual sections. The digital images were analyzed using Image J software. Areas in the intestine and liver structures (*per* 1 mm^2) were analyzed to determine the percentage optical density of positive staining in each region.

RESULTS

Impacts of Alpha-Toc and RA on the histological structure during organogenesis and placentation

Histopathologically, the intestine, liver, and placenta on gestational day (GD) 13.5 revealed that in the normal structure of the control group, the intestine, in which the central epithelial lumen was surrounded by mesenchyme and a smooth muscle layer that consists of longitudinal and



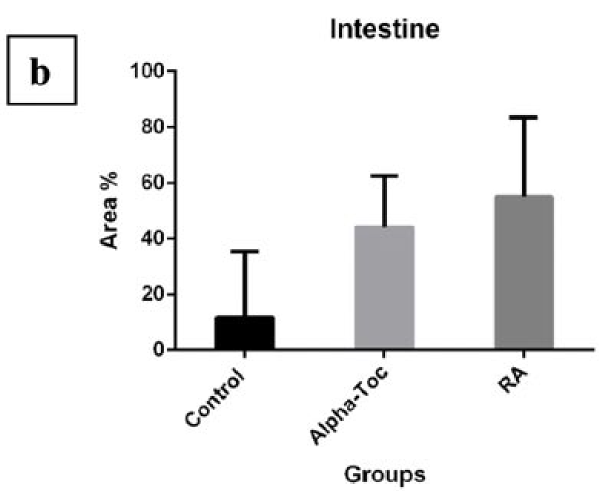


Fig. 1. (a) Photomicrographs of H&E transverse sections of intestine on GD 13.5 of control, Alpha-Toc treated, and RA-treated groups (A-C). Histologically, there are apoptotic cells in the RA-treated group compared to the control group (white arrows). Also, Photomicrographs of immunohistochemical localization of CDKN1B from the control (D), Alpha-Toc treated (E), and RA-treated (F) groups show the expression levels of CDKN1B (red arrows). (b) The histogram represents the optical intensity of CDKN1B-positive signals in the intestinal tissues of alpha-tocopherol (Alpha-Toc) and retinoic acid (RA) treated groups compared to the control group. Density is expressed as the number of positive areas. Internal epithelial layer (IEL) and smooth muscle layer (SML). Scale bar: 10 µm (400X).

circular muscle fibers. The internal epithelial layer was organized in finger-like protrusions, so-called villus structures (Fig. 1a, panel A). Moreover, the liver cells showed typical histological features of normal differentiation and were organized by hepatic strands surrounding the central vein. The liver parenchyma appeared homogeneous and exhibited a uniform distribution of cells. (Fig. 2a, panel A). Furthermore, the placenta became mature and consisted of two parts: the maternal and fetal parts. The maternal part consisted of decidual basalis, and the decidual basalis was composed of a regression of mesometrial decidua, containing decidual cells, fibers, and vascular channels. The fetal part consisted of three layers: the trophoblastic giant cells layer, the spongiotrophoblast layer, and the labyrinth layer. The trophoblastic giant cells, identified by their large nuclei, were present as a layer separating the spongiotrophoblast from the decidual basalis. The spongiotrophoblast layer consisted of large trophoblast cells with small blood sinuses. Both the trophoblastic giant cells and spongiotrophoblast cells formed the junctional zone. The labyrinth layer was formed by contact between the chorionic trophoblast and allantoic mesoderm and contained blood sinusoids (Fig. 3a, panel A). In the Alpha-Toc treated group, our results showed that Alpha-Toc did not affect the development of the intestine, except for the formation of the internal epithelium layer. Also, the cells of the internal epithelium layer were not differentiated completely, resulting in villi deformation compared to the control group (Fig. 1a, panel B). In addition,

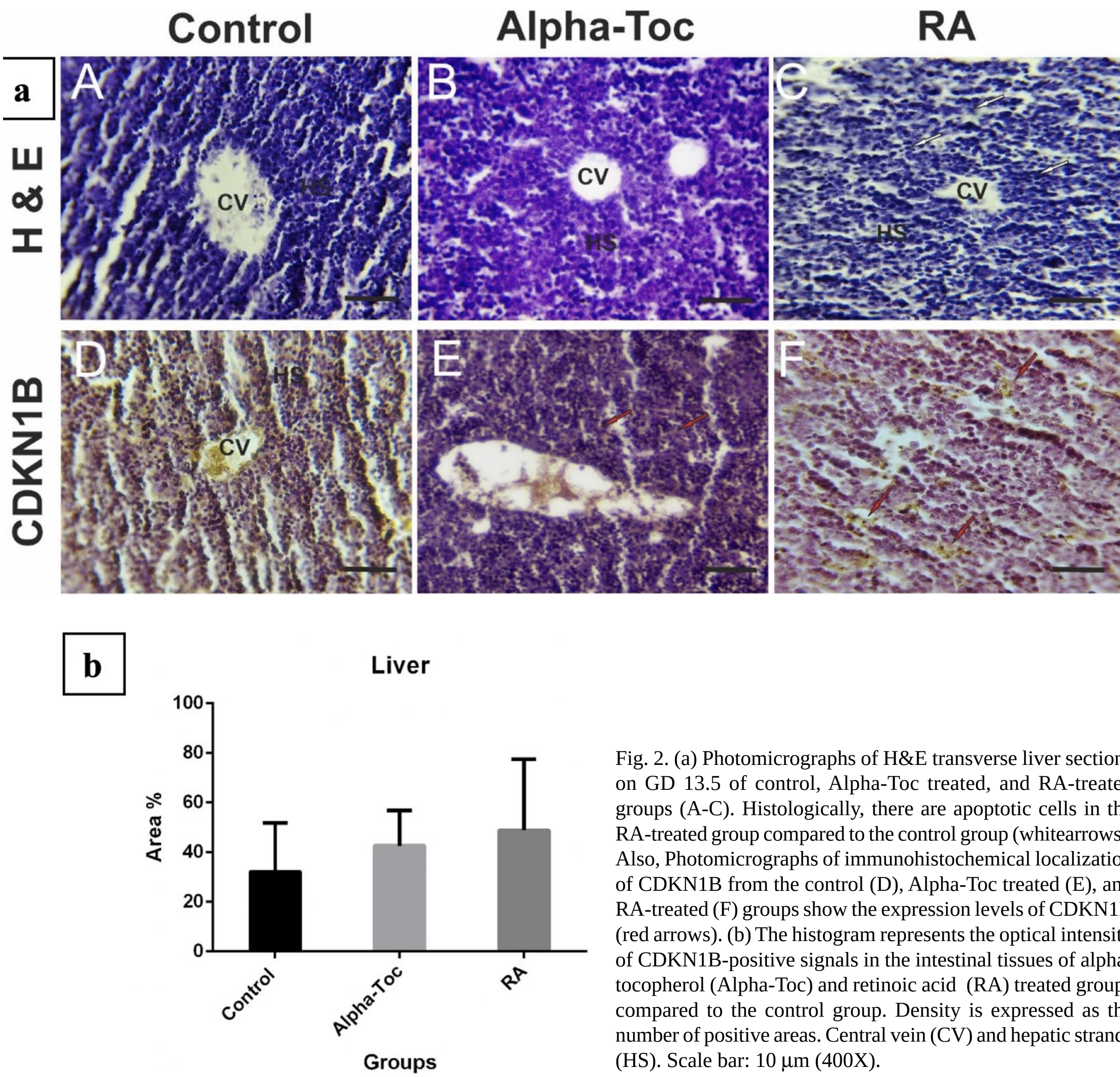


Fig. 2. (a) Photomicrographs of H&E transverse liver sections on GD 13.5 of control, Alpha-Toc treated, and RA-treated groups (A-C). Histologically, there are apoptotic cells in the RA-treated group compared to the control group (whitearrows). Also, Photomicrographs of immunohistochemical localization of CDKN1B from the control (D), Alpha-Toc treated (E), and RA-treated (F) groups show the expression levels of CDKN1B (red arrows). (b) The histogram represents the optical intensity of CDKN1B-positive signals in the intestinal tissues of alpha-tocopherol (Alpha-Toc) and retinoic acid (RA) treated groups compared to the control group. Density is expressed as the number of positive areas. Central vein (CV) and hepatic strands (HS). Scale bar: 10 µm (400X).

the liver transverse sections displayed no alterations in the structure, but there were slightly disorganized hepatic strands compared to the control group (Fig. 2a, panel B). Furthermore, our findings showed that the placenta structure was similar to a control group without any malformations, but there was disorganization in the epithelial cells of the labyrinth zone (Fig. 3a, panel B).

Interestingly, our results showed severe malformations during histogenesis in RA-treated groups compared to a control group. During intestinal development, RA delayed the growth of the ileum, especially the formation of the internal epithelium layer and smooth muscle layer. We showed the disorganization of longitudinal and circular muscle fibers that form the smooth muscle layer and surround

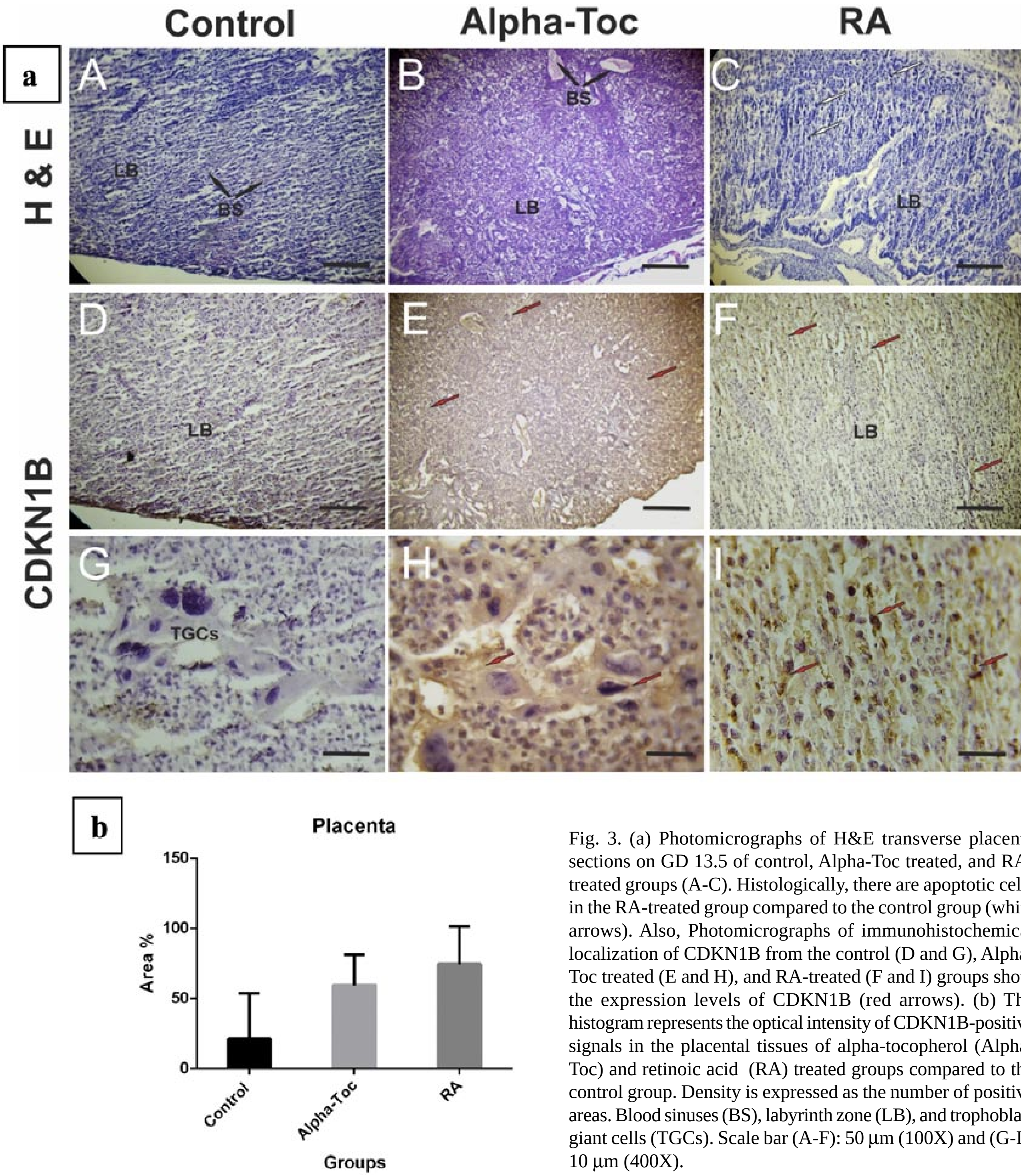


Fig. 3. (a) Photomicrographs of H&E transverse placenta sections on GD 13.5 of control, Alpha-Toc treated, and RA-treated groups (A-C). Histologically, there are apoptotic cells in the RA-treated group compared to the control group (white arrows). Also, Photomicrographs of immunohistochemical localization of CDKN1B from the control (D and G), Alpha-Toc treated (E and H), and RA-treated (F and I) groups show the expression levels of CDKN1B (red arrows). (b) The histogram represents the optical intensity of CDKN1B-positive signals in the placental tissues of alpha-tocopherol (Alpha-Toc) and retinoic acid (RA) treated groups compared to the control group. Density is expressed as the number of positive areas. Blood sinuses (BS), labyrinth zone (LB), and trophoblast giant cells (TGCs). Scale bar (A-F): 50 µm (100X) and (G-I): 10 µm (400X).

the luminal epithelium. Also, the cells of the internal epithelium layer were not differentiated completely, resulting in villi deformation (Fig. 1a, panel C). Moreover, it has been shown that more abnormal histological changes in liver structure. The central veins showed small size and signs of dilation, and there was degradation in the hepatic cells due to apoptosis (white arrows), resulting in highly disorganized hepatic strands. Exhibits an increase in cell density, with cells appearing tightly packed and overlapping. Overall, the liver structure showed severe disruption and disorganization (Fig. 2a, panel C). Furthermore, during placentation, RA delayed the growth of the placenta with defects that ranged from a reduction of the spongiotrophoblast and labyrinth layers to severe labyrinth dysmorphogenesis. RA induced inhibition of the development of both the junctional and labyrinth zones without blood sinusoids, and it also showed apoptosis in trophoblast giant cells and spongiotrophoblast cells of the junctional layer and epithelial cells of the labyrinth layer compared to those of the control group (Fig. 3a, panel C).

Alpha-Toc and RA upregulate CDKN1B immunoexpression

Our study examined the effects of Alpha-Toc and RA on the cell cycle via modulating CDKN1B localization during the intestine, liver, and placenta development. The intestine of the control group showed negative expression of CDKN1B in both the internal epithelium and smooth muscle layers, indicating the continuous differentiation of cells. However, we showed that a strong signal appeared in the serosal mesothelium that surrounded the ileum (Fig. 1a panel D, Fig. 1b). The liver of the control group showed faint expression of CDKN1B in liver parenchyma, especially in hepatic cells that organize the hepatic strands (Fig. 2a, panel D, Fig. 2b). The placenta of the control group revealed that, in the decidual basalis, there was no expression in non-decidualized cells (fibroblasts) and some rounded cells of the central area and close to the smooth muscle coat. In addition, decidualized cells had a negative signal. Moreover, CDKN1B was not expressed in the junctional zone, especially in trophoblastic giant cells and the labyrinth zone (Fig. 3a, panels D and G, Fig. 3b).

On the other hand, the Alpha-Toc treated group of the intestinal structure showed a moderate signal of CDKN1B in the internal epithelium layer compared to the control group. Also, we revealed the strong expression level of CDKN1B in the smooth muscle layer compared to the control group (Fig. 1a, panel E, Fig. 1b). Moreover, in the liver, our findings showed that the expression of CDKN1B was moderate in the hepatic cells compared with the control group (Fig. 2, panel E, Fig. 2b). Furthermore, in the placenta, Alpha-Toc upregulated CDKN1B in the junctional especially in trophoblast giant cells and the epithelial cells of the labyrinth layer, resulting in strong expression compared to a control group (Fig. 3a, panels E and H, Fig. 3b).

In addition, we found during intestinal development that RA upregulated CDKN1B, resulting in strong expression in internal epithelium cells, but in the smooth muscle cells, we observed a moderate expression of CDKN1B (Fig. 1a, panel F, Fig. 1b). Furthermore, in the liver, we showed a strong signal of CDKN1B in hepatic cells (Fig. 2a, panel F, Fig. 2b). Also, in the placenta, we observed that the activatory effect of RA on the expression of CDKN1B was also strongly evident in all placenta layers, resulting in a rise in the expression of CDKN1B in the decidual basalis, junctional zone, especially trophoblastic giant cells, and the epithelial cells of labyrinth zone to a strong level (Fig. 3a, panels F and I, Fig. 3b).

DISCUSSION

The present study investigates the comparative impacts of alpha-tocopherol (Alpha-Toc) as a vitamin E active form and retinoic acid (RA) as a vitamin A active metabolite on the cell cycle during cell proliferation in mouse organogenesis and placentation via activating CDKN1B ($p27^{Kip1}$). Vitamin E is a potent intracellular antioxidant. It is known to inhibit lipid peroxidation and has been demonstrated to have a wide range of anti-cancer properties (elAttar & Lin, 1995). These properties include the inhibition of tumor progression and protection against carcinogenesis. To our knowledge, the precise mechanism of action of vitamin E is unknown. Notably, Fleshner *et al*. (1999) previously showed that vitamin E supplementation can inhibit tumor progression in nude mice bearing human prostate cancer xenografts. Also, previous studies have shown that cell growth inhibition is mediated by various mechanisms (Israel *et al*., 1995; Agarwal, 2000).

The novelty of our study is the follow-up of CDKN1B activation by Alpha-Toc in normal cells during embryogenesis and placentation. Whereas, our findings revealed that Alpha-Toc activates CDKN1B during intestine, liver, and placenta development, indicating that it stops cell proliferation in some tissues during histogenesis. Although Alpha-Toc upregulates CDKN1B, the intestine, liver, and placenta were not completely affected histologically, but there was a little alteration, like deformation of villi in the internal epithelium of the intestine, disorganization of hepatic strands of the liver, and disorganization of the epithelial cells of the placental labyrinth zone. Previously, a candidate tumor suppressor gene, p27, belongs to the Cip/Kip family of cyclin-dependent kinase inhibitors. It has been suggested to have an important role in normal cell cycle regulation and cancer. It regulates the progression of the cell cycle from the G1 to S phase by binding to and inhibiting the cyclin E/ cdk2 complex (Tsihlias *et al*., 2000). Although mutations in the p27 gene appear to be rare in human tumors, alterations in the posttranslational processing of p27, leading to decreased amounts of p27 protein, have been detected in various cancers. Low levels of p27 protein interfere with the ability of cancer cells to halt cell cycling and lead to the accumulation of additional genetic alterations and more malignant growth (Coats *et al*., 1996). Moreover, previous studies reported that vitamin E induces cell cycle arrest in prostate cancer cells, mediated by the up-regulation of p27 (Venkateswaran *et al*., 2002).

In contrast, we observed a slightly normal histogenesis during the development in vivo, suggesting that Alpha-Toc reduces apoptosis according to previous studies, which reported that Alpha-Toc has inhibited apoptosis induced by various stimuli, probably due to its radical scavenging capacity, as many models of apoptosis (programmed cell death, PCD) involve the generation of reactive oxygen species (ROS) (Haendeler *et al*., 1996). Because of its high reducing capacity, Alpha-Toc is a promising physiological candidate for an inhibitor of PCD, as shown in various models of apoptosis. Several groups reported that Alpha-Toc or its analog gamma-tocopherol (Gam-Toc) were active in suppressing apoptosis in endothelial and smooth muscle cells exposed to oxidized low-density lipoprotein (Mabile *et al*., 1995; de Nigris *et al*., 2000). Further studies revealed that in a model of neuronal apoptosis involving early ROS generation, Alpha-Toc efficiently suppressed apoptosis by inhibiting the formation of radicals (Chan *et al*., 1999), and this activity of the drug was confirmed in vivo (Tagami *et al*., 1999).

On the other hand, previous studies revealed that excessive RA during gestation causes abnormalities in human infants as well as in rodents. RA has a teratogenic effect during embryogenesis and placentation by inhibiting the epidermal growth factor receptor (EGFR) signal (Said *et al*., 2022). Also, our findings reported the teratogenic effect of RA on the intestine, liver, and placenta formation by activating CDKN1B, resulting in cell cycle arrest during cell proliferation. In agreement with our results presented here, CDKN1B expression was elevated in most telencephalon regions, particularly in the marginal zone and falx cerebri during brain formation by RA. Elevated CDKN1B expression in proliferative cells led to ventricular zone deformation in the diencephalon's epithalamus and the spinal cord's marginal zone (Said *et al*., 2024).

During histogenesis, we showed apoptotic cells in the studied organs, indicating that RA not only arrests the cell cycle but also can induce apoptosis, according to previous reports. The effects of RA on apoptosis, proliferation, migration, and invasion have been reported in Cholangiocarcinoma (Chung *et al*., 2012). In addition, an earlier report demonstrated that upregulating retinoic acid receptor beta (RARB) in Cholangiocarcinoma tissue enhanced apoptosis in Cholangiocarcinoma and thus improved Cholangiocarcinoma chemotherapeutic sensitivity (Ren *et al*., 2016). Also, retinoids and vitamin A derivatives, cause mitochondrial dysfunction and trigger ROS production to induce cellular damage and apoptosis (de Oliveira, 2015; Oliveira, 2015). Additionally, RA has been reported to induce ROS production in Sertoli cells and NB4 cells (Conte da Frota *et al*., 2006; Miyoshi *et al*., 2010). Moreover, RAs and other retinoid-related compounds cause growth inhibition, accompanied by induction of differentiation and/ or apoptosis, in various types of cancer cells (Bollag *et al*., 1994; Lippman *et al*., 1995). In addition, RA can also act as a potent apoptosis-inducer (Pettersson *et al*., 2000).

The novel finding in this study is that Alpha-Toc and RA induce cell cycle arrest, but we suggested that Alpha-Toc did not induce apoptosis; in contrast, RA induces the presence of apoptotic cells.

CONCLUSION. Our findings concluded that although Alpha-Toc did not affect the histogenesis of the intestine, liver, and placenta during development, we found that CDKN1B was elevated by both Alpha-Toc and RA, resulting in cell cycle arrest. We can say histologically, Alpha-Toc was not a teratogen; in contrast, RA had teratogenic effects and induced cell degeneration.

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RESUMEN: Vitaminas como la vitamina A y la vitamina E se utilizan en numerosos tratamientos y cosméticos. También desempeñan un papel vital en el desarrollo fetal durante la gestación. Por lo tanto, este estudio tuvo como objetivo explorar los efectos negativos de altas dosis de ácido retinoico y alfa-tocoferol durante el desarrollo embrionario y la placentación. Quince ratones preñados se dividieron en tres grupos (n=5/grupo): (1) control (que recibió dimetilsulfóxido (DMSO) diluido con aceite de girasol), (2) ratones tratados con alfa-tocoferol (que recibieron una dosis de 50 mg/kg/día) y (3) ratones tratados con AR (que recibieron una dosis de 10 mg/kg/día). Se administraron inyecciones intraperitoneales diarias con un volumen de 0,1 ml en los días 10,5, 11,5 y 12,5 de gestación. Los animales fueron sacrificados por dislocación cervical el día 13,5 de gestación. Se realizó histología e inmunolocalización de CDKN1B. Histopatológicamente, el intestino, el hígado y la placenta no se vieron afectados por Alpha-Toc; por el contrario, la AR causó malformaciones graves durante la histogénesis de los órganos estudiados en comparación con el grupo control. Inmunohistoquímicamente, nuestros hallazgos revelaron una regulación positiva de la inmunotinción de CDKN1B tanto por Alpha-Toc como por AR en proporciones variables. Se observó que el alfa-tocoferol y el ácido retinoico aumentaron la expresión de CDKN1B, lo que afectó la histogénesis del intestino, el hígado y la placenta, pero a través del ácido retinoico, no del alfa-tocoferol.

PALABRAS CLAVE: Alpha-Toc; Histogénesis; Intestino; Hígado; Desarrollo de la placenta; RA.

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